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Genetic Study on Milled Rice Amylose Content by Means of Single-Grain Amylose Analysis[1]

Yih-Chuan Kuo[2], Bill D. Webb[3], and James W. Stansel[4]

Abstract: Amylose content of milled rice is a significant quality parameter. Genetic analysis of amylose content was conducted using single-grain amylose analysis. Intermediate amylose content to low appeared to be controlled by a single incompletely dominant gene in the combinations between the intermediate and low amylose parents. Two incompletely dominant complementary genes conditioning high over low were suggested from the combinations between high and low amylose parents. A genetic hypothesis of a single incompletely dominant gene controlling high to intermediate amylose was fitted in mating high with intermediate amylose parents. Evidence that amylose content was also influenced by genes of minor effects, or modifiers was also observed in some combinations. The segregation model of endosperm characteristics was illustrated under the assumption of no dosage effect.

Key words: Genetic studies, Milled rice, Amylose content, Single-grain amylose analysis.

INTRODUCTION

Amylose content of milled rice is considered as one of the most important quality parameters for predicting rice eating and processing behavior(13,21,27,28). However, much of amylose data obtained up to 1971 was traditionally used the method reported by Williams et al(28). or its modified procedures(12). These methods for amylose content determination were base upon bulk seed sample with sample size ranging from 100 mg rice flour recommended by Williams et al.(28) to 500 mg used in the studies of Stansel(24). Due to scientist's endeavors and advances in the precision of analytic instruments, a totally automated procedure for analysis of whole grain of milled rice has been innovated(25).

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1. Contribution No. 1826 from Taiwan Agriculture Research Institute.
2. Rice Breeder, Department of Agronomy, TARI., Wufeng, Taichung, Taiwan, ROC.
3. Research Chemist, National Rice Quality Lab., ARS-USDA., Texas A&M University Agricultural Research and Extension Center, Beaumont, Texas.
4. Resident Director and Professor, Texas A&M University Agricultural Research and Extension Center, Beaumont, Texas.

Amylose content of the rice grain has been considered as the result of the interaction between genetic properties and environment factors. Amylose content within a rice cultivar could vary by as much as 6 percentage points[1]. Among the different environment factors affecting amylose content, temperature during grain ripening was the dominant factor [2,5,16,22,24], followed by nitrogen fertilization and the degree of milling[20]. Besides, there was some variation between individual rice plants, between panicles within a plant[7,20], and between individual grains within a panicle[26], with a higher variation between panicles than between individual plants. Thus, the use of bulk samples for amylose determination has been recommended[7,20].

Based upon single-grain amylose analysis, Kumar and Khush[15] indicated that the genes for high amylose content were completely dominant to intermediate or low and amylose content was controlled by a single gene or two complementary genes and modifiers, varying from cross to cross. Dosage effects appeared to be observed, depending on the rice entries evaluated[10,14,15]. However, most of published reports, based upon bulk seed analysis, indicated that high amylose content was incompletely dominant to low and was controlled by one major gene and several modifiers[3,18,23]. The role of two complementary genes was indicated in some studies[8,18,24]. The genetic system deduced from the distribution of amylose content in F_2 populations indicating typical quantitative inheritance was shown in another studies [3]. Transgressive segregation for amylose content was found in some studies[3,18], but not observed in another study[24].

In Taiwan, there have been several published genetic reports in relation to milled rice amylose content[4,9,16,17]. However, in these studies, high×low cross combinations were used as experiment materials and bulk seed sample was analyzed for amylose content determination. So far, the genetic analysis of amylose content by means of single-grain amylose determination has not yet been presented in Taiwan. The objectives of thes report were to introduce the method of single-grain amylose determination to the rice scientists of Taiwan and to present some basic genetic information deduced from high×intermediate, intermediate×low, and high×low amylose combinations.

MATERIALS AND METHODS

F_2 combinations evaluated were from the crosses of mating Lemont with Taichung Sen 10 (TCS 10) and Rexmont with TCS 10, as well as Guichow with Lemont. Among these rice entries, Lemont with intermediate amylose content represents a high quality traditional southern U.S. long-grain cultivar and is now a leading cultivar in the southem rice-producing states of USA. TCS 10, a high yielding indica rice cultivar, was released from Taiwan, ROC rice breeding program in 1975 with a high yielding and quality (in particular low amylose level) as priorities. Rexmont with amylose content 2-4% higher than those of the traditional southern U.S. long-grain cultivars was developed specifically to satisfy the rice industry's need. Guichow, an indica short-grain cultivar with high amylose content, was released from China mainland under a breeding program with high yielding as a priority.

The detailed mating scheme was referred to as listed in Table 1.

Amylose content of a whole milled rice grain was determined by following the method innovated by Webb[25] using a Technicon AutoAnalyser (Technicon Instruments Corp., Tarrytown, NY). The detailed procedures are: on Day 1, a single kernel is weighed and placed into a 25mm × 100mm test tube, and then is soaked overnight in a 2ml 1.7% KOH. On Day 2, 1 ml 2.5N NaOH is added and then agitated vigorously with a Vortex shaker. On Day 3, a single grain sample placed in the test tube is tissumized 30 seconds with a tissumizer at 45-50 rpm and the blade of tissumizer on shaft is rinsed with 17ml distilled water. The sample is then shaken vigorously again with a shaker. On Day 4, The single grain samples are shaken again before starting a run with a Technicon AutoAnalyser. The data obtained are corrected by the grain weight that had been recorded on Day 1.

RESULTS

Mating Lemont with TCS 10

The mean amylose content of the intermediate amylose parent, Lemont, was 23.2% (a total of 40 random milled rice grains) with a range from 21 to 25% and a mode at 23%. The low amylose parent, TCS 10, had a mean of 15% (40 grains) with a range from 12 to 17% and a mode at 15%. A difference in amylose content between the two parents was approximately 8%. The coefficients of variation of the two parents were 4.81% for Lemont and 10.63% for TCS 10, respectively. It is well known that the coefficients of variation of the parents are regarded as an estimate of the environment variation under the condition of the test. Thus, it appeared, form the data mentioned, that environment effect on differentiating amylose content between two parents, Lemont and TCS 10, seemed different slightly. However, the frequency distributions of milled rice amylose content for the parents were similar, and both showed approximately normal distributions (Fig. 1).

The 360 F_2 seeds of the Lemont x TCS 10 combination ranged in amylose content from 12 to 29% with a mean of 22.3% and a coefficient of variation of 19.12%. In contrast, in the F_2 seed population of TCS x Lemont combination had a mean amylose content 22.4% with a range from 12 to 30% and a coefficient of variation of 19.79%. It is well recognized that the coefficient of variation of the F_2 population is a measurement of the total variation, i. e., both environment and genetic variation. In the present case, one could suppose that both environment and genetic variation involved in these two F_2 populations were approximately similar.

The frequency distributions of milled rice amylose content of F_2 seed populations in both combinations exhibited continuous but appeared to be bimodal curves (Fig. 1). Maximum frequency classes were observed at 16 and 23% in Lemont x TCS 10 combination and 16 and 24% in TCS x Lemont combination, respectively. On the other hand, minimum frequency classes were found at 20% in both combinations. According to Juliano[11], amylose content of milled rice is traditionally classified as low (below 20%), intermediate (20-25%), and high (above 25%). Thus, the distributions of two F_2 seed populations could be

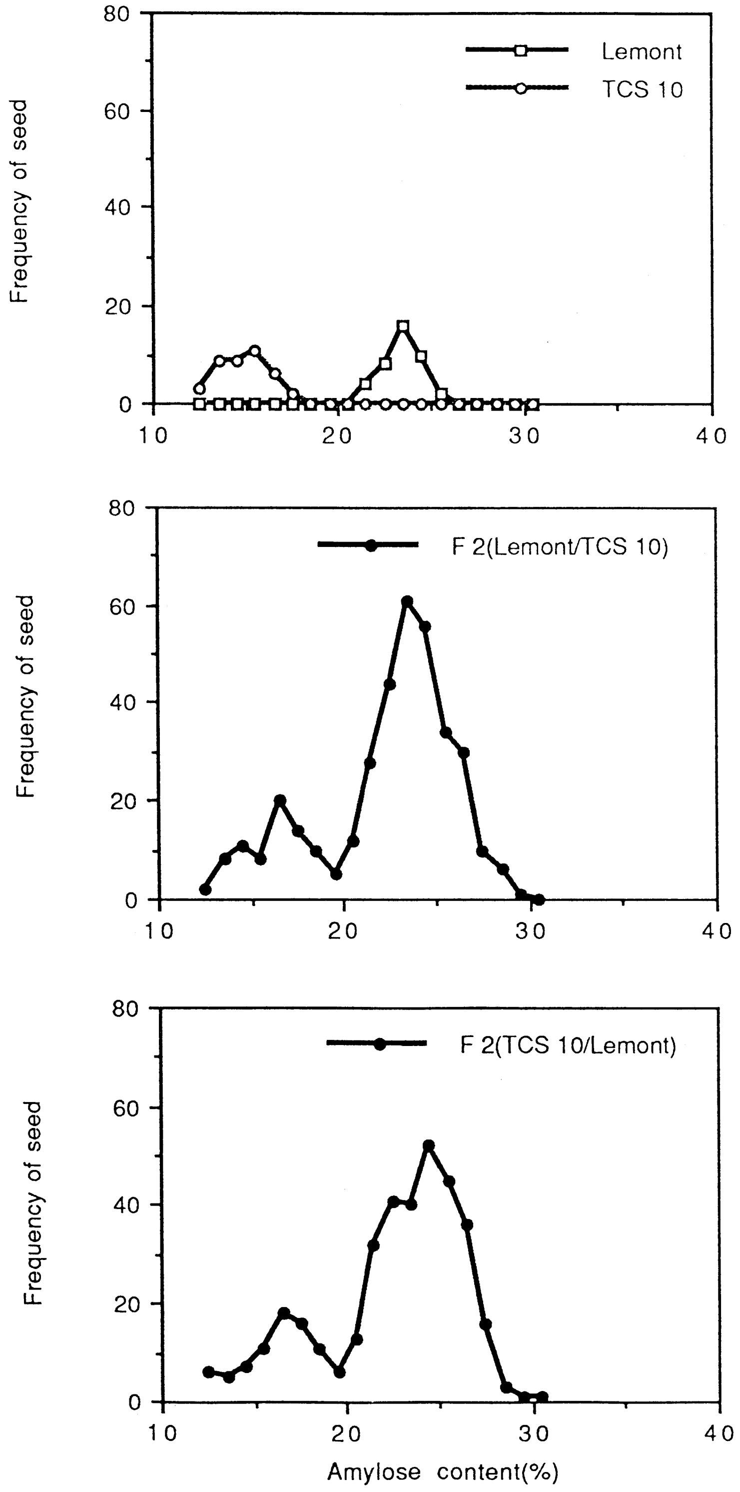

Fig. 1. Frequency curve showing distributions of amylose content in the combinations between Lemont and TCS 10.

categorized into two amylose groups: a low amylose group and an intermediate amylose group with approximately one-fourth of the seeds falling into low groups (78 in Lemont x TCS 10 and 80 in TCS 10 x Lemont combinations, respectively) and three-fourths in the intermediate groups (282 in Lemont x TCS 10 combination and 280 in TCS 10 x Lemont combination, respectively), suggesting segregation of one pair of major genes, with intermediate amylose incompletely dominant. The hypothesis of a single major gene conditioning amylose content in these combinations was further tested. The Chi-square (X^2) values indicated a very good fit to a 3 intermediate: 1 low segregation ratio (Table 1).

Table 1. Chi-square values for test of goodness of fit to genetic segregation models for amylose content of the rice combinations studied.

Combination[z]	Number of F_2 seed	Genetic model	Expected segregation	df	X^2	P
Lemont/TCS 10	360	1 dominant gene	3 : 1	1	2.13	0.20-0.10
TCS 10/Lemont	360	1 dominant gene	3 : 1	1	1.48	0.30-0.20
Rexmont/TCS 10	360	2 complementary genes	13 : 3	1	0.37	0.80-0.70
TCS 10/Rexmont	360	2 complementary genes	13 : 3	1	0.04	0.90-0.80
Guichow/Lemont	180	1 dominant gene	3 : 1	1	2.40	0.20-0.10
F_1/Guichow	100	1 dominant gene	7 : 1	1	0.02	0.90-0.80
F_1/Lemont	100	1 dominant gene	3 : 5	1	1.80	0.70-0.60

In all combinations, the first parent indicates the female parent and the second male.

A larger number of transgressive segregates for high amylose content were observed in both F_2 populations. These would indicated that the low amylose parent, TCS 10, carried, in addition to a pair of genes of major effect for low amylose content, certain modifiers of minor effect for high amylose content[3,4,6]. Therefore, a genetic system of differentiating amylose content in relation to mate Lemont with TCS 10 was controlled by one pair of genes of majoı effect and modifiers of minor effect, and the major gene for intermediate amylose content was incompletely dominant to its allele.

Mating Rexmont with TCS 10

The mean amylose content of the high amylose parent, Rexmont, was 28.1% (40 grains) with a range from 26 to 29% and a mode at 27%. The mean for the low parent, TCS10 was 15.1% (40 grains) showing a range from 12 to 16% and a mode at 14%. Thus, the parent difference in amylose content was about 13%. The coefficients of variation of the parents were 2.10% for Rexmont and 4.90% for TCS 10, respectively. This indicated that the coefficient of variation of Rexmont was about half that of TCS 10. The frequency distributions of amylose content for the parents seemed to be appeared as skewed rectangles and to be differed from a normal distribution as shown in (Fig. 2).

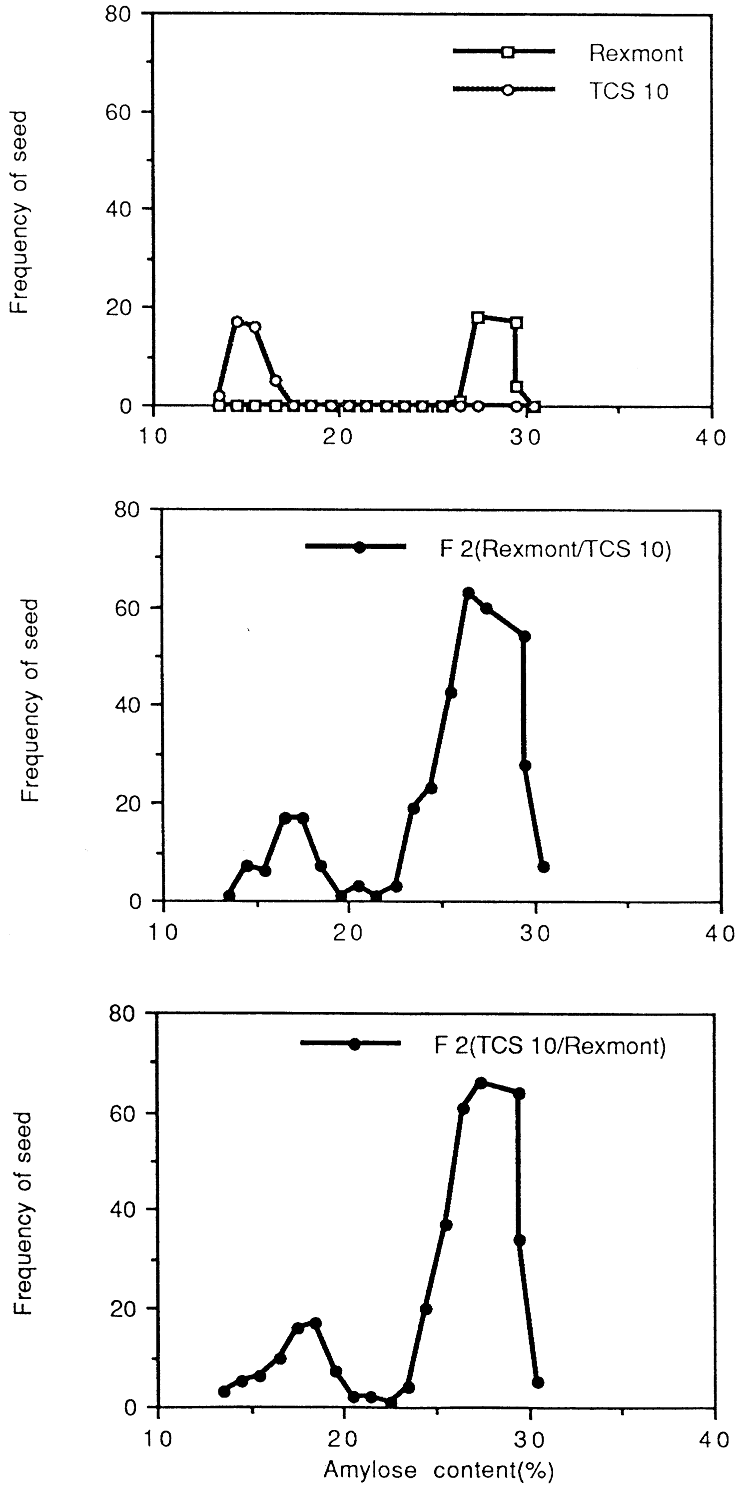

Fig. 2. Frequency curve showing distributions of amylose content in the combinations between Rexmont and TCS 10.

The 360 F_2 seeds in the Rexmont x TCS 10 combination ranged in amylose content from 13 to 30% with a mean 25.2% and a coefficient of variation of 16.19% and that in the TCS 10 x Rexmont combination showed an average amylose content of 25.4% with a range from 13 to 30% and a coefficient of variation of 16.34%, indicating that both environment and genetic variation in these two combinations seemed approximately similar.

The frequency distributions of amylose content of the F_2 seed populations in both combinations formed essentially bimodal curves (Fig. 2). Maximum frequency classes were observed at 17 and 26% in Rexmont x TCS 10 combination and 18 and 27% in TCS X Rexmont combination, respectively. Minimum frequency classes occurred at 21% in Rexmont x TCS 10 combination and 22% in TCS 10 x Rexmont combination, respectively. The F_2 frequency distribution of amylose content in both combinations could be classified into two amylose groups: a low group with approximately three-sixteenths of the seeds (63 in Rexmont x TCS 10 combination and 69 in TCS 10 x Rexmont combination, respectively) and a high group with thirteen-sixteenths of the seeds (297 in Rexmont x TCS 10 combination and 291 in TCS 10 x Rexmont combination, respectively). The results suggested segregation for two complementary major genes, with high amylose incompletely dominant to low amylose. The genetic hypothesis of two complementary genes conditioning high amylose content, which would segregate 13 High: 3 low in F_2 populations, was then tested. The Chi-square (X^2) values supported a satisfactory fit to the 13 high: 3 low complementary gene model (Table 1). That is, a dominant and recessive epistasis was involved in conditioning high amylose in both F_2 populations, i. e., A epistasis to B, b; bb epistasis to A, a. Thus, A_ and bb exhibited identical phenotype assuming A and B were incompletely dominant in conditioning high amylose.

Transgressive segregation for amylose content were not observed in these two F_2 populations.

Mating Guichow with Lemont

The mean amylose content of the high amylose parent, Guichow, was 27.5% (20 random milled rice grains) with a range from 25 to 28% and a mode at 27%. The mean of the intermediate amylose parent, Lemont, was 23.6% (20 grains) having a range from 22 to 24 %and a mode at 23%. Therefore, the difference between the parents about amylose content was approximately 4%. The coefficients of variation of the parents were 2.04% for Guichow and 2.54% for Lemont, respectively. It is simply because the coefficients of variation of the parents are usually regarded as an estimate of the environment variation under the condition of the test. Thus, the environment effects on amylose content for the parents, Guichow and Lemont, appeared approximately similar. Besides, the frequency distribution of amylose content for the parents showed typically normal distributions (Fig. 3).

The 180 F_2 seeds of the Guichow x Lemont combination ranged in amylose content from 21 to 30% with a coefficient of variation of 7.51% and a mean of 26.9%, which was about1.5 % higher than that of the mid-parent 25.5%. The 100 F_2 seeds of F_1 x Guichow combination had a mean amylose content of 27.2% with a range from 21 to 30% and a coefficient of

variation of 6.62%. The 100 F_2 seeds of F_1 x Lemont combination showed an average amylose content of 25.2% with a range from 21 to 29% and a coefficient of variation of 7.38%. These figures suggested that a slight differences in the total variation for amylose content might exist among the three F_2 seed populations. It is well recognizes that the coefficient of variation for the F_2 population is a measurement of both genetic and environment variation. Thus, the slight difference in total variation in the three populations were probably due to genetic variation because that environment effects on amylose content for the parents seemed approximately similar under the condition of the test, i. e., environment effects in the three populations on influencing amylose content, thus, could be considered to be similar.

The frequency distribution of the F_2 seed amylose content in the Guichow x lemont combination was continuous but appeared to be bimodal, with maximum frequency classes at 24 and 28% and minimum frequency class at 25% (Fig. 3). The distribution of the F_2 seed population could be divided into two groups: a high amylose group and an intermediate amylose group, with approximately one-fourth (36 grains) of the seeds falling into the intermediate group and three-fourths (144 grainls) in the high, indicating segregation for one major gene in conditioning high amylose content and incompletely dominant. When testing genetic hypothesie of a single gene pair conditioning high amylose content was done, Chi-square (X^2) value indicated a good fit to a 3 high : 1 intermediate segregation ratio (Table 1).

The frequency distribution of F_2 seed amylose content in F_1 x Guichow combination formed a skewed curve, with the maximum frequency class at 28%. However, as a genetic hypothesis of a single gene pair conditioning high amylose content was postulated, the F_2 population could be categorized into two amylose groups: an intemediate and a high groups, with approximately one-eighth of the seeds falling into the intermediate group (13 grains) and seven-eighths in the high group (87 grains) as dividing at the class 25%, indicating segregation for one major gene pair conditioning high amylose content but being incompletely dominant. When the genetic hypothesis of a single gene was tested, Chi-square (X^2) value indicated a very good fit to a 7 high: 1 intermediate segregation ratio (Table 1).

The F_2 frequency distribution of amylose content in the F_1 x Lemont combination showed a continuous curve but it seemed bimodal (Fig. 3). The genetic hypothesis for the population was also a single gene conditioning high amylose content. Thus, the frequency distribution of the population could also be categorized into two groups: an intermediate amylose group and a high amylose group, with about five-eighths of the seeds falling into the intemediate group (56 grains) and three-eighths in the high group (44 grains), indicating segregation for one major gene with high amylose content incompletely dominant. The Chi-square (X^2) value demonstrated a very good fit to a 3 high: 5 intermediate segregation ratio (Table 1).

There were many F_2 seeds with high amylose content distributed beyond the range of the high amylose parent, Guichow, in the three populations, suggesting that transgressive

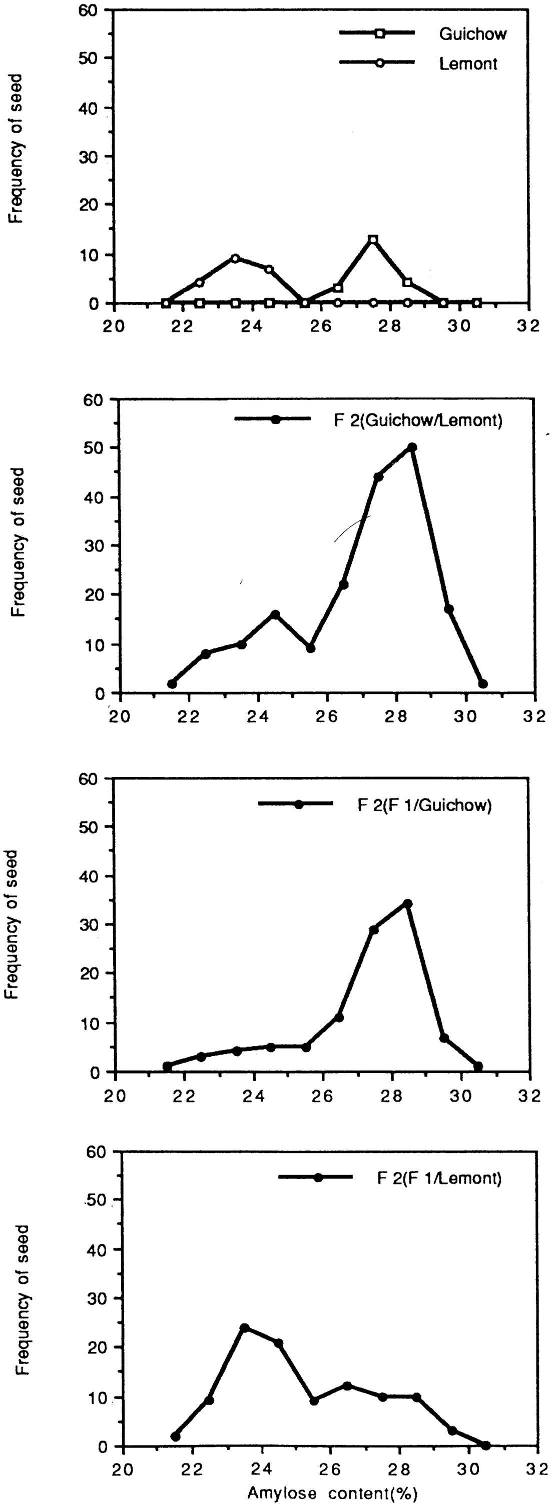

Fig. 3. Frequency curve showing distributions of amylose content in the combinations between Guichow and Lemont.

segregation for high amylose content existed in the populations. This would indicated that the intermediate amylose parent, Lemont, possessed, in addition to a gene pair of major effect for intermediate amylose content and modifiers of minor effect for high amylose content.

DISCUSSION

It is well recognized that an endosperm (grain) is developed from the fusion of two polar nuclei from the female and one sperm nucleus from the male and the occurrence of the fertilized egg is the beginning of a new generation. As the result, the endosperm on a plant represents the next generation, genetic expression for endosperm charzcteristics is under triploid genetic control, and an individual is a grain, not a plant, especifically for concerning the genetic analysis of endosperm characteristics. Because milled rice amylose content, a chemical property of rice starch, is usually considered to be one of the most important attributes for predicting eating, and prosessing quality parameters[13,21,27,28], both traditional bulk seed and single-grain analyses were used to collect the amylose data for the genetic analysis in various rice breding programs. However, so far, many of published genetic studies on amylose content of rice endosperm were traditionally based upon bulk seed analysis of genetically heterozygous plants, i. e., amylose data of F_2 plants were determined by the bulk F_3 endosperm on the F_2 plants, for example.

The detailed genetic information obtained by both amylose determination methods appeared different slightly. Generally speaking, the genetic system deduced from the analysis of bulk seed sample basis indicated that genes conditioning high to intermediate or low or intermediate to low were incompletely dominant. In contrast, Kumar and Khush[15] found that based upon single—grain analysis, genes for high amylose were completely dominant over those for intermediate or low. They argued that analyses of the bulk seed samples of genetically heterozygous plants provided biased information on the inheritance of milled rice amylose content. In their study, when high amylose level parents were mated with low amylose content parents, the frequency distributions of amylose content, based on single-grain basis, for F_2 and F_1 backcrossed to low amylose parent populations showed discontinuous but bimodal curves. Therefore, the genetic hypothesis of a completely dominant gene conditioning high amylose content over low or intemediate amylose level was demonstrated. According to Kumar and Khush[15], the differences in the genetic deductions between most of the published reports and their results is probably due to the bulk seed analysis used in segergating generation (i.e., genetically heterozygous plants) in these genetic studies. In other words, because of segregation for high and low amylose content, bulk seed analysis would give lower values for amylose content in milled rice within each plant. Consequently, complete dominance would appear to be partial or incomplete dominance. However, published data showed that a wide range in values among individual grains on single panicles was observed for various quality characteristics. For example, percent amylose content, based on single-grain basis, of Lemont rice varied from 18 to 27% from which a normal distribution was formed. The mean of thses grains was 22.3%, which was equal to

that of the mean value from bulk sample determinations for amylose content[26]. Also, there is some variation between individual rice plants and between panicles within plants[20]. Thus, data on amylose content collected by Kumar and Khush[15], based on single-grain basis, were undoubtedly confounded by the variation between grains within panicles and between panicles within plants as well as between plants. It is apparent that these differences in the genetic deductions between most of the genetic reports and the report of Kumar and Khush[15] might not be completely explained by the different basis by which the amylose content has been determined. The genetic background of the materials used in an experiment may have also result in different genetic deduction. Thus, the differences in these two genetic deductions could result partially from sample determination basis, i. e., buld seed versus single-grain, and partly from materials having different genetic background.

A genetic hypothesis based on diploid genetic control due to triploid nature of the endosperm is adequate to describe the data of amylose content analyzed by single grain basis as presented in this paper. For example, for a F_1 plant with a Aa genotype for an endosperm characteristic, the probability of possible combinations of polar nuclei in ovule of the plant will be 1 AA: 1 aa and the possible genotypes of the pollens will be 1 A: 1 a. Thus, the possible genotypes of the F_2 seed population will be 1 AAA: 1 AAa: 1 Aaa: 1 aaa. Suppose genotypes of AAA, AAa, and Aaa possess the same phenotype, i. e., no dosage effect, a 3 A--: 1 aaa segrgation ratio will be observed. When a backcross is made between the F_1 and the dominant parent (P1), the possible genotypes will be 1 AA: 1 Aa for the B_1F_1 plants, and 1 AAA: 1 Aaa for the B_1F_1 endosperms. The possible genotypes of the B_1F_1 endosperms will be 1 AAA: 1 (1 AAA: 1 AAa: 1 Aaa: 1 aaa),i.e., 5 AAA: 1 AAa: 1 Aaa: 1 aaa, as the B_1F_1 plants are selfing. Thus, a 7 A--:1 aaa segregation ratio is found. On the other hand, when the F_1 is backcrossed to the recessive parent (P2), the possible genotypes will be 1 Aa: 1 aa for the B_2F_1 plants and 1 AAa: 1 aaa for the B_2F_1 endosperms. The possible genotypes of the B_2F_2 endosperms will be 1 (1 AAA: 1 AAa: 1 Aaa: 1 aaa) : 1 aaa, i.e., 1 AAA: 1 AAa: 1 Aaa: 5 aaa, as the B_2F_1 plants are selfing. Thus, a 3 A--: 5 aaa segregation ratio will be presented. These ratios are then consistent with that of a diploid segregation. Thus, a diploid genetic model in convenience was used to interpret the inheritance of amylose content based on single-grain analysis as presented in this paper. Results obtined showed good fit to the hypotheses made for three amylose content groups studied. Actually, up to now, all published reports, either based on bulk sample determination or by single-grain analysis, all of the genetic deductions were all based on diploid genetic principles even though it is well recognized that genetic expression of endosperm characteristics is under triploid genetic control. However, in this case, dosage effect has to be ignored.

Finally, compared with other plant quantitative characteristics such as plant height, panicle length, etc., a satisfactory quantitative genetic model for endosperm characteristics has not yet been developed although much attention has recently been devoted to the grain quality. The lack of proper genetic models has been an obstacle in studying the inheritance of endosperm characteristics[19]. Therefore, establishing a quantitative genetic models for endosperm characteristics will be of benefit to breeding programs in cereal crops of which

high grain quality is a priority.

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經藉單粒直鏈澱粉分析的白米直鏈澱粉含量之遺傳研究[1]

郭益全[2] 偉伯比爾[3] 史譚索詹姆士[4]

摘 要

白米直鏈澱粉含量是一個重要的品質介量，本研究以單粒直鏈澱粉分析進行白米直鏈澱粉含量之遺傳研究。在中與低直鏈澱粉含量親本之雜交組合中，中直鏈澱粉含量對低含量受一對不完全顯性基因所支配。而在高與低直鏈澱粉含量親本的雜交組合中，指出高含量對低含量受兩對不完全顯性的互補基因所控制。一個高直鏈澱粉含量對中含量係受一對不完全顯性基因所支配的遺傳假說則符合高與中直鏈澱粉含量親本的雜效組合。另，直鏈澱粉含量尚受微效修飾基因影響之證據也在本研究一些組合中被發現。文中曾就無基因劑量效應的前提下，例釋胚乳性狀的分離模式。

關鍵詞：單粒直鏈澱粉分析、白米、直鏈澱粉含量、遺傳研究。

1. 臺灣省農業試驗所　研究報告第 1826 號。
2. 本所農藝系副研究員。臺灣省　臺中縣　霧峰鄉。
3. 美國農部國家稻米品質實驗室暨德州農工大學農業研究與推廣中心研究化學家。德州　玻瑪。
4. 美國德州農工大學農業研究與推廣中心駐場場長暨教授。德州　玻瑪。

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引進落花生野生種原之特性評估[1]

黃惠娟[2] 曹文隆[2] 楊金興[2] 蔡志濃[3]

摘要：本研究主要探討自美國農部（USDA）及印度之國際半乾旱熱帶作物研究所（ICRISAT）引進的12種野生落花生，在臺灣環境下的抗病蟲性(以抗銹病及葉斑病爲主）及農藝特性，期望藉落花生種間雜交方法將抗病蟲基因及有用的農藝特性導入臺灣之栽培品種，擴大栽培種之遺傳變異的基本資料。

在臺中農試所環境下，經一年以上的觀察評估及病原菌接種結果得知，除四倍體野生種落花生 *A. monticola* 以外，其餘11種二倍體野生種均對銹病具有抗性；葉斑病除了 *A. monticola*、*A. stenosperma* 及 *A. batizocoi* 3種外，其餘9種均具有中度以上的抗性。野生種之生長習性爲一年生或以上的匍匐或半匍匐之蔓性莖，分枝最長可達2公尺，植株形態與直立株型的臺灣主要栽培品種有很大差異。籽粒百粒重在5-17g 之間，明顯小於栽培種的47-68g，且均爲單籽粒之莢果，莢喙則明顯較栽培種突出。野生種油分含量達到50%以上的有6個，其中 *A. batizocoi* 及 *A. paraquariensis* 分別爲55.7%及55.9%，蛋白質含量達33%以上的有4個，其中 *A. cardenasii* 及 *A. pusilla* 均爲35.0%，這些野生種之油分及蛋白質含量均較栽培種爲高。野生種 *A. chacoense*、*A. correntina* 及 *A. pusilla* 的初生子房柄爲綠色，其餘9種與栽培種均爲紅綠色或暗紅色。莖色、種皮色及葉部形態，在各野生種之間的變異均較栽培種大，尤其小葉形態變異非常大，且除 *A. batizocoi* 外，其餘之野生種葉面積明顯小於栽培種。

關鍵詞：落花生栽培種、落花生野生種、銹病、葉斑病、抗性。

在作物育種過程中，爲選拔高產、質優及抗病蟲害等優良性狀，若使用少數幾個優良品種爲雜交親本，會造成作物的遺傳變異減小，一旦發生新的病蟲害或環境災害時，易遭受到嚴重的產量損失，所以作物品種間之遺傳變異大小，在作物整個生產體系中是非常重要的。本省落花生育種自民國34年起至84年，在50年間共育成臺南白1號、臺南12號、臺農6號等20個優良品種，但常有重覆使用優良親本之問題，此易導致本省落花生品種之遺傳變異狹小，像以雜交育成的8個品種中，臺南6、7、10、11號及臺農6號與純系選出的臺南白油豆1號均有親緣關係；臺農4、5及6號與西班牙白間亦具親緣關係；另臺南12號與臺南選9號有很近的親緣關係，故引進新的、可利用的種原將可擴大遺傳變異，尤其是使用近緣野生種爲育種材料，更可將自然資源做最大的利用。Halward 等[8,9]以 PCR、RFLP 技術建立落花生分屬於二個栽培種之25個品系及25個二倍體、2個四倍體之野生種的基因圖譜，發現栽培種中變異極小，但二倍體野生種間有非常大的變異，此顯示野生種落花生具有很大的利用價值。

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1. 臺灣省農業試驗所 研究報告第 1827 號。
2. 本所農藝系助理、助理研究員與助理研究員。
3. 本所植病系助理。臺灣省 臺中縣 霧峰鄉。

落花生屬（*Arachis*）以形態學、地理分佈性及雜交親合性的不同，分爲九個節（section）[14]，*Arachis* 節包括四倍體栽培種 *A. hypogaea* （2n=4x=40）及多種具抗病蟲性的二倍體野生種（2n=2x=20），其他六節中也有可利用的抗病蟲基因，另亦有具高油分，高蛋白質或抗旱性較強之野生種原[3,6,7,15-20,22-29]。落花生種間雜交始於1938年，但受制於相異的基因組及倍數性不同所造成的雜交不親和性，早期之研究並無得到雜交植株，直至專家們開始進行染色體觀察及核型分析，並且利用秋水仙素誘導染色體倍加等技術來克服雜交阻礙的研究後[7,11,12,19,21,27]，在1981年時 Stalker 首度獲得一株種間雜交 F_1[22]，此後陸續有學者利用胚培養、子房柄培養技術建立雜交胚之拯救步驟，亦得到以抗葉斑病及根瘤線蟲等抗病蟲植株[4,6,16,17]，但這些複二倍性之種間雜交植株因染色體數不穩定，缺乏活力，並無實際利用價值。1991年 Simpson[18]將不同基因組之二倍體野生種的雜交 F_1 誘導爲四倍體，再與栽培種雜交，所得到之雜交植株稔性提高，但籽粒極小；至1993年 Simpson *et al.*[20] 應用前述方法，將雜交 F_1 再與栽培種親本回交，育成二個分別爲免疫及高抗根瘤線蟲及葉斑病之品系，其農藝性狀已較接近栽培種，百粒重介於兩親本間，但仍不具推廣栽培之條件。

本試驗主要調查自美國農部及印度國際半乾旱熱帶作物研究所引進之12種野生落花生，在臺灣環境下之抗病蟲特性及農藝性狀，以供本省落花生品種改良及其它遺傳相關之研究利用。

材料與方法

一、材 料

(一)野生種落花生：本所引進分屬於三個節的12種野生落花生，其中11種以抗銹病或抗葉斑病爲目標的二倍體野生種，而 *A. moticola* 是惟一感病的四倍體野生種，其基因組、來源地等基本資料如表 1 ，在經國外多年試驗觀察，茲將較具利用潛能的特性列於表 2 。

表 1. 引進野生種落花生之分節、染色體數、基因組及來源地

Table 1. The section, chromosome number, genome and origin of the introduced wild peanut entries[z].

Species	Section	Chromosome No. (2n)	Genome	PI No.	Origin
1. *A. villosa*	*Arachis*	20	AA	298636	Argentina
2. *A. stenosperma*	*Arachis*	20	AA	338280	Brazil
3. *A. batizocoi*	*Arachis*	20	BB	298639	Bolivia
4. *A. paraquariensis*	*Erectoides*	20	EE	262842	Brazil
5. *A. duranensis*	*Arachis*	20	AA	219823	Argentina
6. *A. chacoense*	*Arachis*	20	AA	276235	Paraguaiy
7. *A. correntina*	*Arachis*	20	AA	262808	Argentina
8. *A. spegazzinii*	*Arachis*	20	AA	262133	Argentina
9. *A. monticola*	*Arachis*	40	AA	219824	Argentina
10. *A. cardenasii*	*Arachis*	20	AA	262141	Bolivia
11. *A. rigonii*	*Erectoides*	20	EE	262142	Bolivia
12. *A. pusilla*	*Triseminalae*	20	TT	338449	Brazil

[z] Quoted in Stalker and Moss[28], and Stalker *et al.*[29]

表 2. 引進野生種原可利用之特性

Table 2. The utility characteristics of the introduced wild peanut entries.

Species		Characteristic	Reference
A. villosa	high oil, high protein, drought tolerance.		(3),(4)
	resistant	spider mite[z], corn earworm[y], leafspot[x], lesser cornstalk borer[w], thrip[v].	(5),(25) (28)
	susceptible	peanut stunt virus[u].	
	immunity	potato leafhopper[t].	
A. stenosperma	resistant	rust[s], lesser cornstalk borer, thrip, leafspot, corn earworm, potato leafhopper.	(11),(25) (28)
	susceptible	spider mite.	
A. batizocoi	immunity	rust, thrip.	(4),(25) (28)
	resistant	potato leafhopper, corn earworm, nematode.	
	susceptible	leafspot.	
A. paraquariensis	drought tolerance, early mature.		(25),(28)
	resistant	leafspot, peanut stunt virus.	
A. duranensis	early mature		(3),(25) (28)
	resistant	leafspot, corn earworm, rust, lesser cornstalk borer, tobacco thrip.	
	immunity	peanut stunt virus, potato leafhopper.	
A. chacoense	immunity	rust.	(7),(25) (28)
	resistant	leafspot, potato leafhopper, thrip, corn earworm, lesser cornstalk borer.	
A. correntina	immunity	rust.	(3),(10) (25),(28)
	resistant	potato leafhopper, peanut stunt virus, corn earworm, lesser cornstalk borer, thrip.	
A. spegazzinii	immunity	rust.	(10),(28)
	resistant	leafspot, corn earworm, thrip, potato leafhopper, peanut stunt virus,	
A. monticola	susceptiblee	leafspot, rust, thrip, peanut stunt virus, spider mite, lesser cornstalk borer.	(3),(7) (25),(28)
	resistant	corn earworm, potato leafhopper.	
A. cardenasii	immunity	rust.	(3),(7) (11),(25) (28)
	resistant	leafspot, corn earworm, thrip, lesser cornstalk borer, potato leafhopper, nematode.	
A. rigonii	immunity	potato leafhopper.	(25),(28)
	resistant	corn earworm, spider mite.	
	susceptible	lesser cornstalk borer, peanut stunt virus.	
A. pusilla	immunity	rust, thrip, potato leafhopper.	(25),(28)
	resistant	spider mite, lesser cornstalk borer, corn earworm.	

[z] *Tetranychus urticae*, [y] *Heliothis zea*, [x] *Cercospora arachidicola* and *Cercosporidium personatum*. (early and late leafspot) [w] *Elasmopalpus lignosellus*, [v] *Frankliniella fusca*, [u] *Frankliniella fusca*, [t] *Empoasca fabae*, [s] *Puccinia arachidis*,

(二)四倍體栽培種落花生

A. hypogaea：臺農 5 號（TN 5）、臺農 6 號（TN 6）、臺南選 9 號（TNS 9）、臺南11號（TN 11）、臺南12號（TN 12）

二、方 法

(一)性狀調查：

種原引進後在溫室及田間進行繁殖，性狀調查記錄之方式主要參考國際植物遺傳種原委員會（IBPGR）[12] 所訂定之格式，另爲配合本省落花生育種目標及栽培狀況，增加調查以下性狀：

1. 葉長及葉寬：全展成熟葉10片頂小葉之平均長度及最大寬度。
2. 莢果長度及寬度：20個成熟莢果之平均長度及最大寬度。

3. 籽粒長度及寬度：20個成熟籽粒之平均長度及最大寬度。
4. 花朵大小：10朵全開花之旗瓣最大寬度。
5. 油分及蛋白質：先將種子磨粉，以130±2°C烘乾 1 小時後，經由 IA 360型之紅外線分析儀（NIR）測定之。

㈡病蟲害檢定：分爲五等級

(1)感染，S=susceptible (2)中抗，MR=moderately resistant

(3)抗，R=resistant (4)高抗，HR=high resistant (5)免疫，I=immunity

1. 田間觀察：整個生育期均進行。
2. 室內接種：以銹病及葉斑病爲主，病原菌孢子在室內培養繁殖後，以孢子濃度爲20,000個/ml的溶液，於檢疫溫室內噴灑植株，10～14天後視其感染葉片面積百分比來判斷。

結　果

一、植株性狀

野生種中除了 *A. paraquariensis* 及 *A. chacoense* 爲半匍匐性（半直立）外，其餘10種均爲主莖及側枝全匍匐或僅是主莖直立而側枝呈匍匐（*A. rigonii* 及 *A. pusilla*）的生長型態，其莖枝花型有 *A. cardenasii*、*A. rigonii* 爲主莖、側枝連續開花型態，其餘均爲主莖開花或不開花，而側枝不連續開花之型態。莖色，在野生種中變異頗大，從綠色、綠帶淡紅、綠帶暗紅及全暗紅色均有。*A. stenosperma*、*A. chacoense*、*A. rigonii* 及 *A. pusilla* 的莖茸毛極稀疏，其餘野生種的茸毛密度從普通到密集均有。在臺灣的環境下野生種之生活史，除 *A. batizocoi* 及 *A. paraquariensis* 爲一年生外，另10種野生種均爲多年生（表 3）。

表 3. 栽培種及引進野生種落花生之植株性狀

Table 3. The plant and agronomic characteristics of cultivated and introduced wild peanuts.

Species	Growth[y] habit	Flowering[x] pattern	Stem color	Stem hairiness	Life form
A. hypogaea[z]	6	2	Green with slight red	Abundant to Moderate	100-140days
A. villosa	1	4	Green with slight red	Abundant	Perennial
A. stenosperma	1	4	Green with dark red	Scarce	Perennial
A. batizocoi	1	3	Green with slight red	Moderate	Annual
A. paraquariensis	4	3	Green with slight red	Abundant	Annual
A. duranensis	1	3	Green or dark red	Abundant	Perennial
A. chacoense	3	4	Dark red	Scarce	Perennial
A. correntina	1	3	Green	Moderate	Perennial
A. spegazzinii	1	3	Green with slight red	Abundant	Perennial
A. monticola	1	4	Green	Abundant	Perennial
A. cardenasii	1	2	Green	Abundant	Perennial
A. rigonii	2	2	Green with slight red	Scarce	Perennial
A. pusilla	2	4	Green with dark red	Scarce	Perennial

[z] Cultivated peanut, including TN5、TN6、TNS9、TN11 and TN12.

[y] 1=Procumbent, but main stem erect. 2=Procumbent. 3=Decumbent, but branch slight erect. 4=Decumbent, but branch moderate erect. 5=Decumbent. 6=Erect.

[x] 1=No flower on main stem, branch alternate.
2=Main stem and branch sequential flowering.
3=Irregular flowering on main stem and branch.
4=Irregular flowering on branch, without flowers on main stem.

二、葉部性狀

野生種之小葉形態的變異非常大，除 *A. chacoense* 爲細小的披針狀及 *A. pusilla* 爲小的倒卵形外，其餘一個野生種內就有多種形態，且常有主莖與分枝小葉形不同之區分，像 *A. duranensis* 主莖小葉形爲小的窄橢圓形，分枝葉形爲小的卵形或倒卵形，*A. correntina* 主莖葉形爲長形，分枝爲橢圓形及卵形，*A. spegazzinii* 主莖葉形爲小的橢圓形，分枝爲小的寬橢圓形及倒卵形；葉形較特殊的有 *A. batizocoi* 之近圓形及圓形葉，*A. paraquariensis* 的長披針狀及披針狀小葉。葉片顏色最深的爲 *A. paraquariensis* 及 *A. pusilla*，最淡的爲 *A. batizocoi* 及 *A. duranensis*，另 *A. stenosperma* 及 *A. speqazzinii* 的葉色爲不太均一的黃綠色。葉長則只有 *A. paraquariensis* 平均達 5cm 以上，與栽培種相近，最短爲 *A. duranensis* 之1.6cm，其餘野生種葉長在2.9～2.1cm。葉寬以 *A. batizocoi* 之 2.2cm 最寬，最窄爲小披針狀的 *A. chacoense* 之 0.6cm，其餘在1.8～1.2cm 之間。小葉長寬比率愈大則葉形愈細長，反之則爲屬於闊葉形，屬於前者的有 *A. paraquariensis*，其比率爲4.0以及比率爲3.8的 *A. chacoense* 均爲披針葉形類，其餘野生種及栽培種屬於闊葉形。葉片茸毛茂密的有 *A. batizocoi* 等 5 個野生種（表 4）。

表 4. 栽培種及引進野生種落花生之葉部性狀

Table 4. The leaf characteristics of cultivated and introduced wild peanuts.

Species	Leaflt[z] shape	Leaf color	Leaflet length (cm)	Leaflet width (cm)	Leaf rate l/w	Hairiness of leaflets
A. hypogaea						
TN5	4,10	Green	6.0±1.06	3.0±0.43	2.0±0.16	Sparse
TN6	4,10	Dark green	5.3±0.10	2.6±0.25	2.0±0.23	Sparse
TNS9	4,10	Slight green	5.2±1.14	2.8±0.31	1.7±0.34	Sparse
TN11	10	Slight green	4.8±0.73	2.3±0.15	2.1±0.20	Sparse
TN12	10	Slight green	4.9±0.73	2.7±0.22	1.8±0.19	Sparse
A. villosa	M : 6 B : 6,10	Green	1.9±0.16	1.2±0.13	1.6±0.12	Sparse
A. stenosperma	M : 11 B : 5,9,11	Yellow green	2.5±0.45	1.3±0.13	2.0±0.22	Sparse
A. batizocoi	7,8	Slight green	2.6±0.53	2.2±0.37	1.2±0.27	Profuse
A. paraquariensis	12,13	Dark green	5.2±0.53	1.3±0.66	4.0±0.83	Profuse
A. duranensis	M : 5 B : 9,10	Slight green	1.6±0.31	1.1±0.17	1.4±0.25	Profuse
A. chacoense	14	Green	2.3±0.14	0.6±0.07	3.8±0.49	Sparse
A. correntina	M : 11 B : 3,9	Green	2.1±0.26	1.2±0.14	1.6±0.20	Sparse
A. spegazzinii	M : 3 B : 6,10	Yellow green	2.1±0.26	1.8±0.25	1.2±0.25	Sparse
A. monticola	6,7	Green	2.9±0.53	1.6±0.29	1.9±0.14	Profuse
A. cardenasii	3,10	Green	2.2±0.42	1.5±0.14	1.5±0.33	Sparse
A. rigonii	9,10	Green	2.1±0.20	1.6±0.27	1.4±0.25	Sparse
A. pusilla	10	Dark green	2.1±0.25	1.3±0.13	1.6±0.24	Profuse

[z] M=Main stem, B=Branch, 1=Cuneate, 2=Obcuneate, 3=Elliptic, 4=Oblong-elliptic, 5=Narrow-elliptic, 6=Wide-elliptic, 7=Suborbicular, 8=Orbicular, 9=Ovate, 10=Obovate, 11=Oblong, 12=Oblong-lanceolate, 13=Lanceolate, 14=Linear-lanceolate, 15=Other

三、莢果性狀

野生種均爲一粒種仁之單粁粒莢果，但亦偶見一子房柄上有兩個各自分開的單粁粒莢果；莢殼網紋則介於中度至輕微之間。莢果最大的爲 *A. paraquariensis*，其莢長及莢寬各爲 1.7cm 及 0.6 cm，而莢果最小的爲 *A. pusilla*，長寬僅有 0.8cm 及 0.4cm。另野生種莢果有一特徵即具較明顯的莢喙（pod beak），一般栽培種莢喙均已退化至不明顯的形態（表 5）。

表 5. 栽培種及引進野生種落花生之莢果特性

Table 5. The pod characteristics of cultivated and introduced wild peanuts.

Species	No. of seeds per pod	Pod reticulation	Pod length (cm)	Pod width (cm)	Pod beak
A. hypogaea					
TN5	2±1	Moderate	2.7±0.18	1.1±0.10	Slight
TN6	2±1	Moderate	4.0±0.51	1.4±0.17	Slight
TNS9	2±1	Slight	2.6±0.11	1.2±0.08	Slight
TN11	2±1	Moderate	3.4±0.25	1.3±0.12	Slight
TN12	2±1	Slight	3.0±0.54	1.2±0.11	Slight
A. villosa	1	Moderate	1.2±0.19	0.6±0.12	Prominent
A. stenosperma	1	Slight	1.6±0.20	0.5±0.02	Very prominent
A. batizocoi	1	Slight	1.4±0.20	0.5±0.07	Very prominent
A. paraquariensis	1	Slight	1.7±0.23	0.6±0.14	Very prominent
A. duranensis	1	Moderate	1.2±0.22	0.6±0.19	Prominent
A. chacoense	1	Slight	1.1±0.14	0.5±0.07	Moderate
A. correntina	1	Slight	1.0±0.13	0.5±0.07	Moderate
A. spegazzinii	1	Slight	0.9±0.10	0.5±0.10	Moderate
A. monticola	1	Moderate	1.2±0.15	0.6±0.09	Very prominent
A. cardenasii	1	None	0.8±0.10	0.5±0.07	Prominent
A. rigonii	1	Slight	1.2±0.13	0.5±0.08	Prominent
A. pusilla	1	Slight	0.8±0.15	0.4±0.09	Prominent

四、粁粒性狀

野生種同一種內粁粒顏色常有2—3種，粁粒長及寬分別在1.3～0.6cm 及 0.5～0.3cm 的範圍內。*A. paraquarinensis* 百粒重 17.3g 爲最大，最小爲 *A. pusilla* 只有 5g，另 *A. cardenasii* 亦只有 5.6g。油分高於50%的有 *A. batizocoi*、*A. paraquariensis*、*A. spegazzinii*、*A. rigonii*、*A. monticola* 及 *A. pusilla*，其中 *A. paraquariensis* 達到55.9%，蛋白質含量較高的四個爲 *A. cardenasii* 之 35.9%、*A. pusilla* 之35.1%、*A. villosa* 之 34.1%及 *A. speqazzinii* 之33.5%（表 6）。

表 6. 栽培種及引進野生種落花生之籽粒性狀

Table 6. The seed characteristics of cultivated and introduced wild peanuts.

Species	Seed color	Seed length(cm)	Seed width(cm)	100 seed weight(g)	Oil (%)	Protein (%)
A. hypoga						
TN5	Rose	1.4±0.12	0.7±0.07	51.1±2.76	50.4	30.4
TN6	Rose	1.8±0.24	0.8±0.13	68.3±5.12	49.3	30.7
TNS9	Rose	1.4±0.11	0.8±0.09	47.1±1.97	50.2	30.2
TN11	Rose	1.6±0.15	0.9±0.10	65.2±3.55	53.5	29.5
TN12	Rose	1.6±0.14	0.8±0.16	58.4±2.27	47.3	32.0
A. villosa	Fresh, Wine, Brown	0.9±0.16	0.4±0.14	12.6±0.50	47.8	33.2
A. stenosperma	Wine, Brown	1.3±0.11	0.4±0.08	14.8±1.12	49.0	29.8
A. batizocoi	Fresh, Wine, Brown	1.0±0.22	0.4±0.99	13.2±0.75	55.7	30.0
A. paraquariensis	Fresh, Wine, Brown	1.0±0.19	0.3±0.09	17.3±0.91	55.9	29.7
A. duranensis	Fresh, Wine, Brown	1.0±0.14	0.4±0.09	9.1±0.25	48.5	32.4
A. chacoense	Fresh, Wine, Brown	0.7±0.12	0.3±0.08	6.0±0.20	48.4	32.4
A. correntina	Fresh, Wine, Brown	0.8±0.15	0.3±0.14	6.5±0.32	49.0	32.4
A. spegazzinii	Fresh, Wine, Brown	0.6±0.09	0.3±0.08	7.0±0.25	50.2	33.5
A. monticola	Wine, Brown	1.0±0.11	0.5±0.08	10.6±0.20	50.1	32.6
A. cardenasii	Fresh, Wine, Brown	0.7±0.10	0.4±0.09	5.6±0.32	45.7	35.0
A. rigonii	White, Wine, Brown	0.8±0.14	0.4±0.07	7.5±0.06	52.7	31.7
A. pusilla	White, Wine, Brown	0.6±0.11	0.3±0.16	5.0±0.25	50.0	35.0

五、花器性狀

A. duranensis 及 *A. pusilla* 的花最小，旗瓣寬度只有1.0cm 及 1.1cm，另 *A. batizocoi* 及 *A. cardenasii* 爲1.3cm，其餘寬度在1.5～1.9cm 之間，接近栽培種的 1.6cm。野生種中除橘色花外，亦有旗瓣爲黃色的黃色花。*A. chacoense*、*A. correntina* 及 *A. pusilla* 之初生子房柄顏色爲綠色，另 9 種以綠色中帶有紅色或暗紅色（表 7）。

表 7. 栽培種及引進野生種落花生之花器性狀

Table 7. The flower characteristic of cultivated and introduced wild peanuts.

Species	Flower standard		Peg color
	width(cm)	color	
A. hypogaea[z]	1.6±0.2	Orange	Green with red
A. villosa	1.6±0.1	Orange	Green with red
A. stenosperma	1.5±0.1	Yellow	Green with dark red
A. batizocoi	1.3±0.1	Yellow	Green, or with red
A. paraquariensis	1.9±0.2	Orange	Green with dark red
A. duranensis	1.0±0.1	Orange	Green
A. chacoense	1.9±0.2	Orange	Green
A. correntina	1.9±0.2	Orange	Green with red
A. spegazzinii	1.6±0.1	Yellow	Green with red
A. monticola	1.8±0.1	Orange	Green with red
A. cardenasii	1.3±0.1	Yellow	Green with red
A. rigonii	1.7±0.1	Yellow	Green with dark red
A. pusilla	1.1±0.1	Orange	Green

[z] *A. hypogaea* including TN5、TN6、TNS9、TN11、TN12.

六、病蟲害觀察與檢定

引進臺灣後所進行的病蟲害田間觀察及室內病原菌接種試驗結果列於表 8，在田間調查易感葉斑病的有 *A. batizocoi* 及 *A. monticola* 二種，而經室內病原菌接種葉片後，則發現還有 *A. stenosperma* 感病。在田間及室內易感銹病的僅有 *A. monticola*，其餘野生種均對銹病具有抗性。受紅蜘蛛危害程度在各種原間差異較大，*A. villosa*、*A. batizocoi* 及 *A. rigonii* 不受危害或危害程度極小，其餘受到的危害較爲嚴重。另蠕蟲（worm，夜蛾類之幼蟲）吃食葉片的情形僅在 *A. stenosperma*、*A. rigonii*、*A. monticola* 及 *A. speqazzinii* 上發現有少數危害。

表 8. 12個引進野生種落花生在田間之抗病蟲性調查及室內病原菌接種結果
Table 8. Pest and disease resistance of the introduced wild peanut in Taiwan.

Species	Leafspot[z]		Rust[y]		Spider mite[x]	Worm[x]
	field	inoculation	field	inoculation		
A. villosa	MR[w]	MR	R	R	1	1
A. stenosperma	MR	S	R	R	7	5
A. batizocoi	S	S	HR	R	1	1
A. paraquariensis	R	R	R	R	3	1
A. duranensis	R	R	HR	R	7	1
A. chacoense	R	R	R	R	5	1
A. correntina	R	R	R	R	3	1
A. spegazzinii	R	R	R	R	3	5
A. monticola	S	S	S	S	5	5
A. cardenasii	MR	MR	HR	R	3	1
A. rigonii	R	MR	R	R	1	3
A. pusilla	R	R	R	R	3	1

[z] level (infect percent in a leaf)＝HR：O, R：1～2, MR：3～4, S：5～9, HS：>10
[y] level (infect percent in a leaf)＝HR：O, R：0.1～3, MR：3.1～5, S：5.1～10, HS：>10
[x] Rating, S＝susceptible；MR＝moderately resistant；R＝resistant；HR＝high resistant.
[w] level, 1＝Very low or novisible sign of susceptibility. 3＝Low. 5＝Intermediate. 7＝Hight. 9＝Very high.

討　論

落花生起源於南美，變異中心在巴西 Mato Grosso 地區[24,26]，許多證據顯示栽培種落花生（基因組爲 AABB），由具 AA 基因組之 *A. cardenasii* 及 BB 基因組之 *A. batizocoi* 天然雜交而成之野生異質四倍體演化而來[13,21,23,28]，經幾世紀的馴化，栽培種植株形態與野生種已大不相同，像臺灣目前栽培品種除澎湖一、二號爲側枝呈匍匐的 Virginia 型外，其餘爲植株直立的 Spanish 型，花之旗瓣色亦只有橘色，不似野生種有黃色花(表 7)。試驗中野生種除 *A. paraquariensis* 及 *A. chacoense* 爲半匍匐性外，另10種均爲分枝可達1～2公尺長的蔓生匍匐性，於臺灣 5 月到10月期間溫度較高時，花開繁盛且植株生長茂密，加上已成熟種子繼續發芽生長，故整個植株覆蓋面可達半徑約 1 公尺的圓形面積，此具有發展爲庭園草皮之潛力。而12個野生種在臺灣均爲一年生或以上，覆蓋力強，像 Stalker *et al*[29]即認爲野生落花生有良好之固氮作用，可發展爲牧草及覆蓋作物。葉部、莢果及籽粒的形態大小，野生種均明顯小於栽培種，即使是與栽培種同爲四倍體的 *A. monticola* 亦無法表現出多倍性植株的巨大形態特性。黃[1]分析250種落花生品種之蛋白質及油分含量，發現品種間有極大的差異（蛋白質17.07～31.94%，油分49.64～63.19%），並建議利用雜交育種方式來改良本省落花生之品質，而本試驗野生種蛋白質及油分則普遍高於栽培種，尤其 *A. spegazzinii* 及 *A. pusilla* 蛋白質含量高外，油分亦達50%(表 6)，實可利用爲育種材料，在 Cherry[5]報告亦曾指出部

份野生種具高油分及高蛋白質之利用潛能。另野生種莖色、小葉葉形、葉色、籽粒色及初生子房柄顏色，在各種野生種間及同一野生種之變異性較大，但籽粒色通常會受成熟後之收穫時間延遲的影響。

銹病及葉斑病爲臺灣重要的病害，目前的栽培品種均不具抵抗性[2]，一旦在溫度、濕度等適宜發病的環境條件下，往往造成重大的損失，雖然目前以藥劑防治，但在成本的提高，環境的污染及防治效果的考慮下，育成具有抗病之新品種實爲刻不容緩。表 2 中引進之種原以抗葉斑病及銹病爲主，像 *A. batizocoi* 等11種野生種對銹病免疫或具抗性，*A. villosa* 等 8 種對葉斑病具抗性，另 *A. villosa* 顯示出具高油分、高蛋白質含量及抗旱性佳，*A. paraquariensis* 亦具抗旱性且較早熟，另 *A. monticola* 爲易感病蟲之種原，主要作爲對照。以在臺灣與國外均進行之銹病、葉斑病及紅蜘蛛的調查結果比較（表 8），不同之處爲 *A. stenosperma* 在國外爲抗葉斑病，引進臺灣後爲易感葉斑病，原因可能在於臺灣之環境下的病原菌生理小種（physiological races）不同所致，另國外的資料中有些種原具有對銹病免疫能力，但經試驗病原菌接種結果均無免疫力。*A. pusilla* 原爲抗紅蜘蛛，而由試驗的觀察得知，在此環境下會受到紅蛛蜘的危害。另在田間不噴施殺蟲劑情況下，栽培種葉片嚴重受到夜蛾類幼蟲危害，野生種除 *A. stenosperma*、*A. batizocoi*、*A. spegazii* 及 *A. rigonii* 受到輕微危害外，其餘均不受影響。在1993年 Yang *et al.*[30]曾指出野生種對夜蛾類及薊馬的抗性與植株表皮油脂質所含的成份有關，而此種抗性的差異是否會受不同環境條件所影響，則有待進一步的探討。另需注意在國外的試驗報告中所調查之蟲害與本省所發生之害蟲，僅紅蜘蛛（*Tetranychus*）爲同屬，但不同種（species），另薊馬、夜蛾類及葉蟬等均爲不同屬的種類[2]。

野生種 *Arachis* 節中之 *A. villosa*、*A. stenosperma*、*A. batizocoi*、*A. duranensis*、*A. chacoensis*、、*A. correntina*、*A. spegazzinii*、*A. monticol*、*A. ipaensis* 及 *A. glabrata* 與 *Erectoides* 節之 *A. rigonii* 等11種均有直接或間接與栽培種雜交成功獲得雜交植株的實例[4,9,11,15,18,23,27]，除 *A. ipaensis* 及 *A. glabrata* 外，其餘 9 種及 *A. paraquariensis*、*A. cardenasii*、*A. pusilla* 本所均已引進觀察，而由以上試驗結果可歸納出野生種在臺灣之可利用特性有(1)抗銹病及葉斑病，(2)抗蟲性，(3)具高油分或高蛋白質含量，(4)具做爲夏季庭園草皮之潛力，(5)可發展爲牧草或覆蓋作物，而針對前三項特性，野生種若能與本省栽培品種進行雜交，再利用染色體倍加及胚培養等技術以獲得稔性高且具活力之雜交植株後，並配合 *PCR* 等生物技術以確定引入希望基因後，再行與栽培種回交，應能培育出抗病蟲之品種。

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Evaluation of Agronomic Characteristics for the Introduced Wild Peanut[1]

Huey-Jiuan Huang[2], Wen-Long Tsaur[2], Kin-Hsing Yang[2] and Jhi-Nong Tsai[3]

Summary

Twelve wild peanuts in three sections of the *Arachis* were introduced from USDA and ICRISAT for interspecific hybridization to broaden the genetic diversity and to improve the disease resistance for the cultivated peanuts in Taiwan. The preliminary results of pathogen inoculation and agronomic characteristics were summarized as follows : Except for *A. monticola* (tetraploid), all the tested wild peanuts were resistant to rust (*Puccinia arachidis*). Nine of the twelves wild peanuts such as, *A. villosa, A. paraquariensis,* etc. were at least moderately resistant to leafspot (*Cercospora arachidicola* and *Cercosporidium personatum*), however *A. stenosperma, A. batizocoi* and *A. monticola* were susceptible. The growth habit of wild peanuts was annual and/or perennial, with procumbent or decumbent vine stems, and the branch crawal up to two meters showed significant different to the cultivars of Taiwan. The 100-seed weight of wild peanuts (5-17g) were less than thoses of the cultivated varieties (47-68g). Pod of the wild species bears only one seed and has prominent beak on the pod. The wild peanuts showed higher oil and protein contents as compared to the cultivate varieties. Six of the twelve wild peanuts contained over 50%, among them *A. batizocoi* and *A. paraquariensis* were 55.7% and 55.9%, respectively. Four of the wild peanuts carry more than 33% protein. Pegs of *A. chacoense, A. correntina,* and *A. pusilla* were green color, however, the peg color of the other wild peanuts and the cultivated varieties were green with red or dark red. A wide variation was found in stem color, seed coat color, and leaf shape of the wild peanuts, especially the leaflet shape. Except for *A. batizocoi,* all the wild peanuts showed smaller leaf as compared to the cultivated varieties.

Key words : Cultivated peanut, Wild peanut, Rust, Leafspot, Resistance.

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1. Contribution No. 1827 from Taiwan Agricultural Research Institute.
2. Respectively, Assisant, Assistant Agronomist and Assistant Agronomist, Department of Agronomy, TARI.
3. Assistant, Department of Plant Pathology, TARI. Wufeng, Taichung, Taiwan, ROC.

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金山地區春夏作甘藷不同植期對塊根產量之影響[1]

賴永昌　廖嘉信　陳一心[2]

摘要：本試驗在1992年進行，主要目的在探討金山地區春夏作甘藷在不同植期下其塊根產量表現情形，試驗結果顯示：甘藷塊根產量隨著生育日數遞增而增加，至植後120天達到最高，在生育期間影響塊根肥大主要氣候因子爲日夜溫差。

關鍵詞：不同植期、日夜溫差。

臺灣地處熱帶和亞熱帶之間，氣候溫暖，極適合甘藷種植，故甘藷爲本省主要雜糧作物之一。一般而言，本省雖周年適合種植甘藷，但不同栽植期，因氣候條件變化，其生育發育、產量及品質等，往往有相當大的差異。本省目前甘藷栽培以秋裡作爲主，根據1994年臺灣省年報統計，秋作及裡作種植面積達70％，而春夏作僅佔30％，造成甘藷周年季節性生產量不均的現象，市場價格差異亦極大，而本省春夏作甘藷種植期間爲高溫、多雨等氣候條件，因此在此環境條件下，對甘藷生育過程中，乾物質之生產、累積、分配等之變化情形及塊根產量構成因素之差異，進行研究瞭解，再配合栽培技術改進，以提高春夏作甘藷的產量。

影響春夏作甘藷生長、產量之氣候及生理因素，目前可供參考之報告並不多，根據李氏（1981）試驗結果發現，春夏作甘藷，在全生育期中，一般莖葉產量較塊根產量爲多，而秋裡作塊根產量比莖葉產量高，此係由於不同期作生育期間氣候條件不同所。甘藷生育期中，適當生育溫度約在20～30°C之間，全期間適當降雨量爲400㎜，若雨量過多，則土壤水分過多，通氣性不良，塊根肥大必受到影響。另日夜溫差亦爲影響甘藷塊根肥大之主因，因日間溫度較高時，能促進光合作用之加強，夜間溫度較低時，能夠抑制呼吸作用，減少養分消耗，故日夜溫大時，有利塊根生長發育及有機養分累積，促進塊根肥大(5)，Wardlaw 等氏（1983）亦發現低夜溫能促進碳水化合物累積，王氏（1985）亦曾探討日夜溫度對落花生產量的影響，發現若日夜溫差較大時增加落花生產量，但在甘藷方面則尙未發現相關報告提出。

收穫指數亦被認爲與塊根產量有密切關係，李氏（1986）從143個品種中發現，塊根乾物重與收穫指數有密切的複迴歸關係存在，塊根乾物重隨收穫指數及總乾物重之淨迴歸及淨相關係數均爲極顯著正數，李氏等（1991）指出甘藷收穫指數與塊根乾物產量之相關爲顯著正相關，且在變動的環境下，其所表現之趨向皆一致。又 Bhagsari and Harmon 兩氏（1982）研究16個甘藷品種，發現在全生育期中，收穫指數與塊根產量之相關爲顯著之正相關。

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1. 臺灣省農業試驗所　研究報告第 1828 號。
2. 本所嘉義農業試驗分所助理研究員、前副研究員、研究員。臺灣省　嘉義市。

本試驗之目的，爲探討春夏作甘藷在不同植期莖葉及塊根生產變化情形，並測定其生育期間氣候條件，以了解春夏甘藷生長之氣候條件之變異對甘藷生育的影響，作爲春夏作生產技術改進之參考。

材料與方法

以甘藷品種臺農66號爲試驗材料，於1992年春夏作在臺北縣金山鄉進行田間試驗，試驗前作爲綠肥，試驗田區土壤經分析爲砂質土，PH 值平均爲5.9，有機質平均爲2.35%，有效磷每公頃平均爲149.3公斤，有效鉀肥每公頃平均357公斤（表1）。試驗設計採用逢機完全區集設計，種植時期爲試驗處理，分別於4月、5月及6月種植，小區爲8行區，行長10公分，行距100公分，株距25公分，重複5次。每公頃肥料施用量，氮肥80公斤，磷肥40公斤及鉀肥160公斤，分2次施用，第一次在種植時，施氮及鉀肥之半量及磷肥之全量作爲基肥，第2次於植後30天左右，將剩餘氮及鉀肥之半量作爲追肥施用。

於植株生育至60天、90天及120天時，分別進行取樣，每次自每一小區逢機取樣10株，區分爲地上部莖、葉及地下部塊根等，並將樣品於送入乾燥箱中以70°C連續烘乾24小時，秤量乾物重，調查鮮莖葉產量、乾莖葉產量、鮮塊根重、乾塊根重、每個塊根平均重、地下莖節數及其直徑等，及並於生育期間調查雨量、溫度及日夜溫差等氣候條件，作爲瞭解春夏作甘藷之栽培環境。

結果與討論

一、不同種植期生育期間氣候條件之差異比較

圖1、2、3爲1992年臺北縣金山地區各月份之平均日溫（°C）、每月累積雨量（mm）及月平均日夜溫差（°C）之變化。4月、5月及6月植全生育期分別爲4月至8月、5月至9月及6月至10月，在這三種生育期之月平均日溫皆高於20°C以上，差異不並明顯（圖1），月累積雨量亦介於229～305 mm，亦無明顯差異（圖2），但月平均日夜溫差則發現4月植全生育期爲5.7至7.5°C之間，尤其在塊根肥大時之生育後期分別爲6.7°C、7.5°C及6.5°C，而5月及6月植甘藷生育後期月平均日夜溫差則偏低（圖3），故日夜溫差爲本期甘藷生育期間較明顯差異之氣候條件。

表 1. 1992年度春夏作甘藷生長調查田區土壤分析

Table 1. The soil fertility analysis in Jin-Shan 1992 spring and summer season

Area	Date	Soil texture	PH	Organic matter (%)	Phosphorus (kg/ha)	K_2O (kg/ha)	CaO (kg/ha)	MgO (kg/ha)
Jin Shan	81.4.17	sandy loam	6.3	2.45	174	397	6.400	1.420
Jin Shan	81.4.20	sandy loam	6.2	2.09	137	357	5.770	1.620
Jin Shan	81.5.15	sandy loam	5.3	2.50	137	317	3.920	1.120

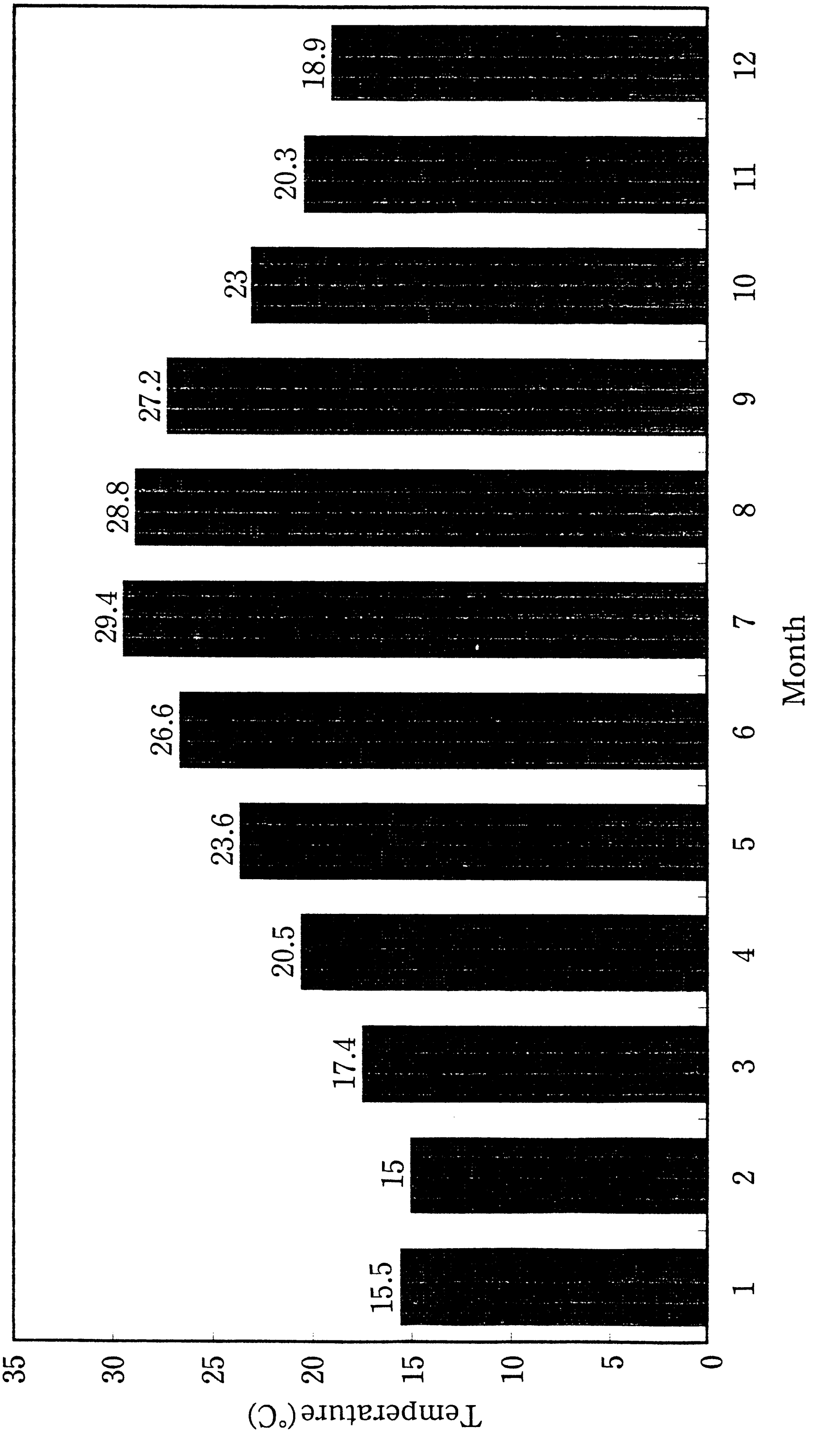

圖 1. 1992年在金山地區每月平均日溫變化情形

Fig. 1. Daily mean temperature (°C) at Jin Shan area in 1992.

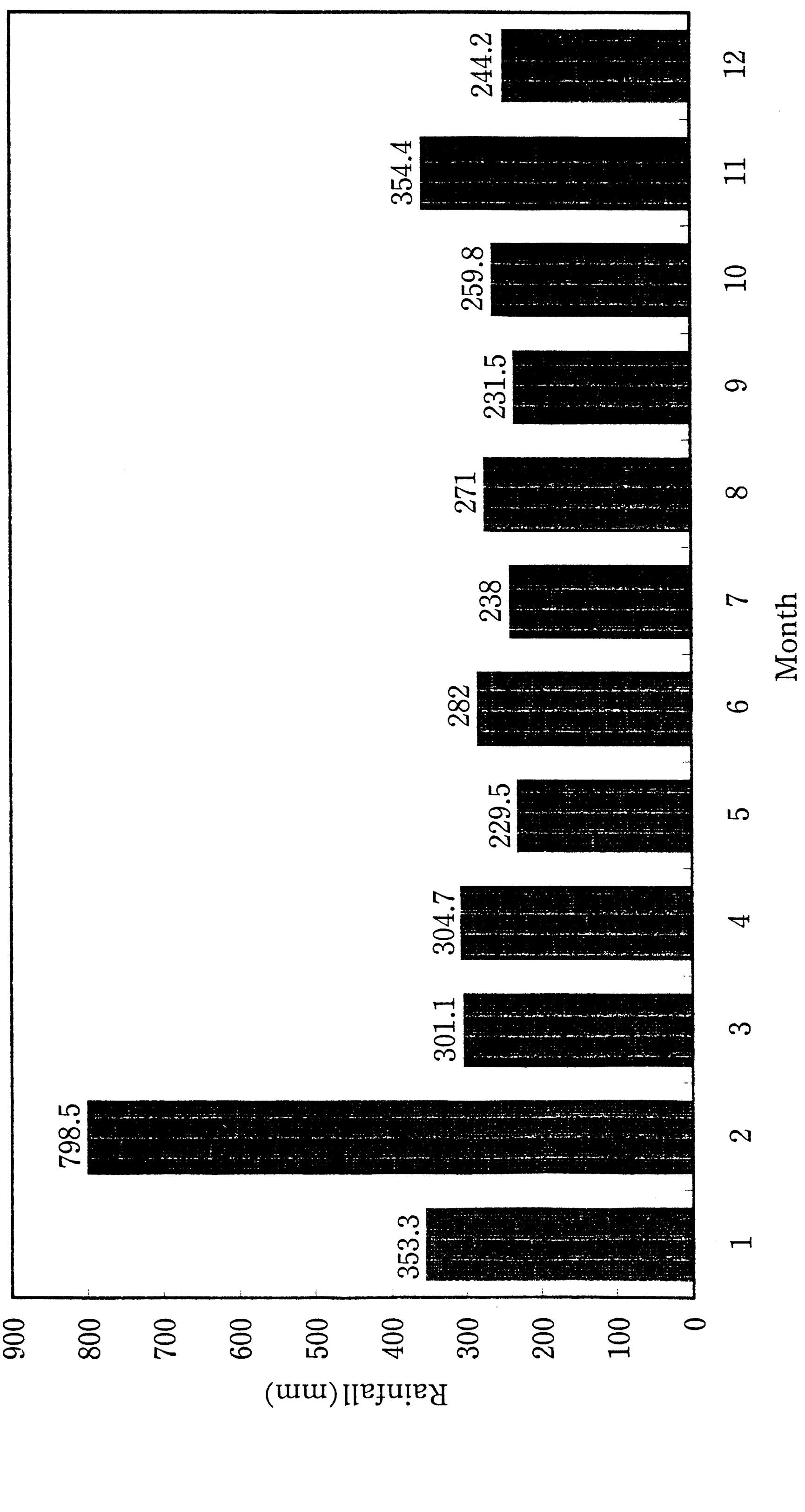

圖 2. 1992年在金山地區每月累積雨量變化情形

Fig. 2. Monthly rainfall (mm) at Jin Shan area in 1992.

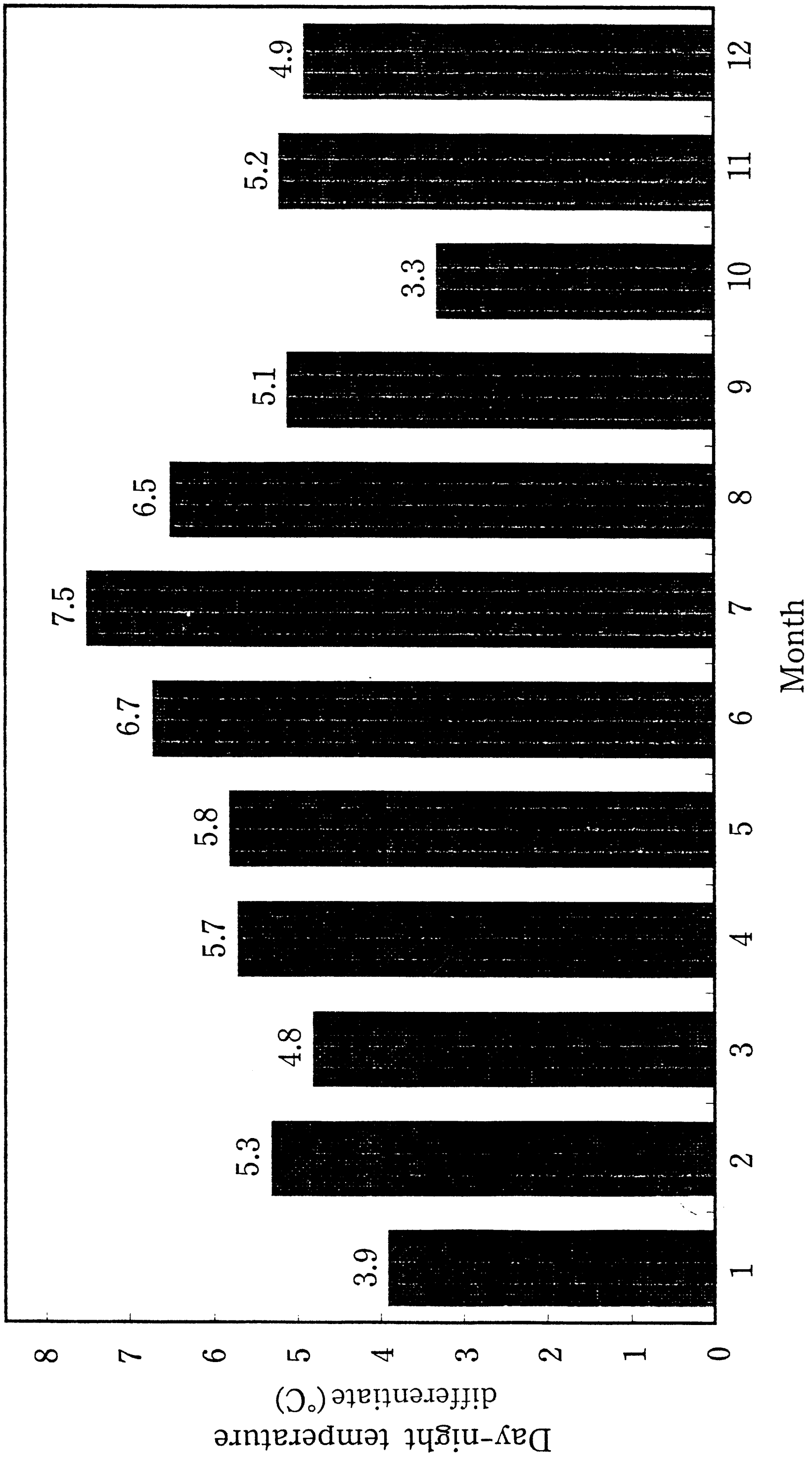

圖 3. 1992年在金山地區每月日溫差變化情形

Fig. 3. Day-night temperature differentiate at Jin Shan area in 1992.

二、不同種植期甘藷莖葉及塊根之生長比較

1992年金山地區種植期分別4、5及6月之甘藷臺農66號在生育期60天、90天及120天所測定莖葉及塊根產量變化情形，由表2、3、4結果得知，不同月植之甘藷莖葉及塊根皆隨生長時間而增加，4月、5月及6月植甘藷莖葉產量皆於植後90天皆達到最高量，其後即開始下降；而塊根產量4月、5月及6月植皆於植後120天達到最高量。此乃因甘藷插植後至90天之生長期爲植株地上部營養生長旺盛期，自生育90天後，光合產物分配於塊根之比率隨生育日數之增加而增多，而光合產物分配於莖葉之比率，隨生育日數之增加而減少，促使莖葉產量降低而塊根產量增加，此結果與李氏等[2,6,10]研究甘藷生育期間光合產物生產及分配之結論相同。再比較不同月植莖葉與塊根產量，發現4月植甘藷植後120天塊根產量達每公頃31,230公斤，每株乾物量亦達280公克，較於植後90天達最高點之莖葉產量爲高，而5月及6月植甘藷植後120天塊根產量皆較莖葉產量最高量之生育期低。李氏[3]曾以爲本省春夏作甘藷塊根產量皆較莖葉產量低，但金山地區4月植甘藷塊根產量反而較莖葉產量高，顯示4月植甘藷對光合產物分配於塊根之效率較5、6月植爲佳，比較三種不同月植生育期間之氣候條件，發現生育期間月平均溫度皆高於20°C（圖1），皆利於甘藷植株生長[5]，但差異並不明顯；再比較其雨量，發現三種月植全生育之累積雨量雖皆高於400mm（圖2），但金山地區甘藷種植地勢較高之山坡地，排水效果良好，故對甘藷生長並無明顯影響；再比較三種月植之月平均日夜溫差，則發現4月植甘藷生育期間之日夜溫差明顯較其他月植甘藷之日夜溫差爲高（圖3），李（1994）曾認爲日夜溫差加大時有利甘藷生長發育及有機養分累積，促進塊根肥大，Warldlaw 等氏（1983）亦認爲低夜溫能促進碳水化合物累積，王氏（1985）則認爲日夜溫差提高能增加落花生之產量，故4月植甘藷生育後期日夜溫差高，利於甘藷塊根生長及養分累積，因而促使塊根產量亦提高，而5及6月植甘藷生育後期月平均日夜溫則較低（圖3），故造成4月植甘藷塊根產量較莖葉產量高，5、6月植之莖葉產量高於塊根產量，換言之，以甘藷塊根產量而言，金山地區一年當中最適宜栽培期應在4月份種植。

表 2. 比較金山地區甘藷臺農66號各生育期莖葉、塊根重及收穫指數之變化情形

Table 2. Comparison of weight of tops, weight of sterage roots and harvest index in three growing periods of sweet potato variety" TNG 66" at Jin-shan area.

Character	Days after planting		
	60	90	120
Fresh yield of tops (kg/ha)	13,890±4,542	15,975±3,767	15,355±5,021
Dry matter yield of tops (g/plant)	47.0±11.3	70.0±16.8	72.0±17.3
Fresh yield of storage roots (kg/ha)	3,300±799	13,290±3,218	31,230±5,524
Dry matter yield of Storage roots (kg/ha)	27.0±72.0	120.0±32.1	280.0±74.8
harvest Index (HI, %)	36.5	63.2	79.5

Planting date: 81.4.20

表 3. 比較金山地區甘藷臺農66號各生育期莖葉、塊根重及收穫指數之變化情形

Table 3. Comparison of weight of tops, weight of sterage roots and harvest index in three growing periods of sweet potato variety ″ TNG 66″ at Jin-Shan area.

Character	Days after planting		
	60	90	120
Fresh yield of tops (kg/ha)	12,920±7,913	31,280±14,654	12,070±8,095
Dry matter yield of tops (g/plant)	70.0±48.7	125.0±86.9	61.0±42.4
Fresh yield of storage roots (kg/ha)	6,596±2,936	26,962±12,003	27,846±9,061
Dry matter yield of Storage roots (kg/ha)	51.0±21.9	224.0±96.4	232.0±99.8
harvest Index (HI, %)	42.1	64.2	79.2

Planting date: 81.5.15

表 4. 比較金山地區甘藷臺農66號各生育期莖葉、塊根重及收穫指數之變化情形

Table 4. Comparison of weight of tops, weight of sterage roots and harvest index in three growing periods of sweet potato variety ″ TNG 66″ at Jin-shan area.

Character	Days after planting		
	60	90	120
Fresh yield of tops (kg/ha)	23,760±7,448	33,012±8,718	6,192±2,440
Dry matter yield of tops (g/plant)	96.0±19.9	99.8±20.8	48.0±9.9
Fresh yield of storage roots (kg/ha)	4,050±1,269	10,573±3,315	22,932±7,189
Dry matter yield of Storage roots (kg/ha)	28.1±8.2	79.9±23.4	171.0±49.9
harvest Index (HI, %)	22.6	44.5	78.1

Planting date: 81.6.20

收穫指數被認為與甘藷塊根產量呈正相關[3,4,7,8]，故收穫指數之高低可作為決定適當採收期之指標。由於表2、3、4顯示金山地區4、5及6月植甘藷之收穫指數皆隨著生育時間而增加，至植後120天達最高，且塊根產量亦隨收穫指數增加而遞增，收穫指數之高低亦可被認為甘藷塊根產量高低之指數，作為決定採收時期之依據，此項結果與李氏[2]研究不同期作間，收穫指數一般以生育初期較低，生育中、後期較高，及李氏等[3,4,7]及 Bhagsari and Harmon 兩氏[8]認為收穫指數與塊根產量呈正相關之結論相同。表2、3、4亦發現4、5及6月植甘藷於植後120天，收穫指數皆已達78～80%之間，皆已達採收期，換言之，金山地區4、5、6月植甘藷種植120天（4個月）即達適當採收期。

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Effects of Different Planting Dates on the Storage Root Yield of Spring and Summer Crop Sweet Potato in Jin-Shan Area[1]

Yung-chang Lai, Chia-Hsin Liao and Yi-Sin Chen[2]

Summary

The effects of different planting dates on the Storage root yield of spring and summer season sweet potato in Jin-Shan was studied in 1992. Results indicated that the storage root yield of sweet potato increased markedly during growth period and reached the maximun at 120 days after planting. Day-night temperature diffenentiate is the most important climatic factor to affect the enlargement of storage root of sweet potato.

Key words : Sweet potato, Different planting dates,
Day-night temperature differentiate.

1. Contribution No. 1828 from Taiwan Agriculture Research Institute.
2. Respectively, assistant agronomist, former associate researcher and sinor agronomist . Chiayi Agricultural Experiment Station, TARI. Chiayi, Taiwan, ROC.

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甘藍及青花菜的花藥培養

I.品系、發育期及培養基與再生之關係[1]

張有明　劉邦基　蕭吉雄[2]

摘要：甘藍初秋、和風及高峰三品種進行不同發育期的花藥培養；結果顯示花蕾在0.4公分以下（約晚單核期前）培養所誘導癒合組織率高。青花菜和甘藍各11品種（系）行花藥培養，培養基採用 MS 配方，另加2,4-D 和 BA 不同濃度組合。甘藍於液態培養時的癒合組織生成率比固態培養高，唯癒合組織較光滑、鬆散；而品種間差異極大，癒合組織生成率介於 0 ～47.3%。青花菜品種間癒合組織生成率差異也頗大，但固態或液態培養則差異不明顯。生長調節劑 BA 濃度爲0.5ppm 時，不論液態或固態培養皆有利於甘藍及青花菜癒合組織的生成；2,4-D 濃度在癒合組織的誘導上，品種間反應不一，唯其平均之效果均以0.5 ppm 和1 ppm 較佳。將0.5公分以上大小的癒合組織，移至含有0.5ppm BA 和三種 NAA（4 0，0.5，1 ppm）濃度的分化培養基中，經由間接器官形成途徑，青花菜和甘藍共有 4 品種（系）已獲得再生植株。移植前先培養於含有蛭石和珍珠石之介質（取代洋菜），並促進根系健全發育，可以提高組織培養苗移植到田間的成活率。植株經根尖體檢定，已知部份爲單倍體植株。

關鍵詞：青花菜、甘藍、花藥培養、單倍體。

十字花科蕓苔屬爲非常歧異化的（diversity）植物，同時大多爲自交不親合，遺傳組成呈高度異結合性[16]。本屬植物由於用途廣泛而成爲世界性之經濟作物[26]。在本省栽培的種類很多，如芥菜、結球白菜、小白菜、芥藍、青花菜、花椰菜和甘藍等，均爲重要之經濟作物[1]；其中甘藍和青花菜的栽培面積更高達一萬公頃以上[4]，全年均有栽培，爲本省重要的蔬菜之一。

單倍體或同質結合之雙倍體材料，在作物遺傳、育種研究上極具價值[3,5,9]。由於蕓苔屬的青花菜和甘藍爲異交作物，傳統方法需較長時間才能育成同質結合之自交系；且在選育過程中，有明顯的自交弱勢現象，造成選拔上極大之困擾；不利於同質結合自交系之培育，影響育種工作甚鉅[18]。若能以花藥培養的方法，產生單倍體或自然倍加之同質雙倍體植株，對蕓苔屬高純度自交系的獲得及一代雜交種之育成極爲重要[2,10,11]。報導指出西洋油菜（*Brassica napus*）[12]、中國白菜（*B. campestris* ssp. *pekinensis*）[23]和青花菜（*B. oleracea* L. var. *italica*）[14,22]等，均能經由花藥培養產生單倍體，而且多數是以胚發生（embryogenesis）的路徑產生單倍體植株。已知花藥培養能否成功，受基因型的影響極大[20]；因此本研究擬以不同來源的甘藍（*B. oleracea* L. var. *capitata*）和青花菜（*B. oleracea* L. var. *italica*）品種（系），進行花藥培養，探討不同的基因型及培養基，誘導花藥培植體再生植株之效率。

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1. 臺灣省農業試驗所　研究報告第 1829 號。
2. 臺灣省農業試驗所園藝系助理研究員、副研究員、研究員兼系主任。臺灣省　臺中縣　霧峰鄉。

材料與方法

一、供試材料：

收集美國、日本及國內的青花菜和甘藍地方品種、商業品種及部份農試所的雜交後代（表1）。播種於溫室，俟植株莖粗0.5～1公分左右移至光照16小時5～8°C低溫的生長箱，行春化處理4至8週，秋冬天則利用天然低溫（臺大 梅峰農場，海拔約2,000公尺左右），以獲得大量花蕾。於抽苔開花時，取田間或生長箱之花蕾，經消毒後再進行培養。

表 1. 甘藍和青花菜花藥培養供試品系

Table 1. The tested materials of cabbage and broccoli for anther culture

variety	generation	source	
cabbage			
初秋	F_1	Takii	
純系-3	S_2	91-302	
(夏峰1號×巴西-2) F_5→op	op	91-087	
(PI281552×初秋) ⊗	F_8	91-112	
(PI281552×初秋) F_7⊗→op	op	91-116	
(PI281552×初秋) F_7⊗→op	op	91-118	
〔(PI281552×60日)×初秋〕⊗	F_8	91-130	
〔(PI281552×$F_1$107)F_4〕op→⊗	S_4	91-131	
〔(PI281552×$F_1$107)F_4〕op→⊗	S_4	91-151	
〔(PI281552×$F_1$107)F_4〕op→⊗	S_4	91-177	
〔(PI281552×$F_1$107)F_4〕op→S_3→op	op	91-185	
broccoli			
綠王	F_1	農友公司	
綠芳	F_1	農友公司	
Broccoli-mikamo	F_1	農協行	TVI6665
Broccoli	F_1	第一園藝	TVI6666
綠嶺	F_1	Sakada	TVI6667
Gulicome greencorn	F_1	Takii	TVI6669
Hai Tzu	F_1	Takii	TVI6670
Baiolo Tokuin	F_1	Takii	TVI6671
Calaxy	F_1	Asgrow	TVI6723
Neptune	F_1	Royal Sluis	TVI6728
XPH5004	F_1	Asgrow	TVI6734

二、培養條件：

將大小不同的花蕾取下，以75%酒精浸泡30秒，再放到超音波振盪器中，用稀釋五倍的 chlorox 浸泡20分鐘，每次浸泡後均以無菌水清洗三次，於無菌臺上剝取花藥，置於不同的培養基中。培養時先以37°C高溫暗處理24小時，再移至適溫（26～28°C）不照光下進行培養，此方法乃參考 Keller 和 Armstrong (1983)[14]並稍加修正。每週調查一次花藥及癒合組織生長和分化的情形。

(一)不同蕾期試驗：用初秋（Chu Chou, K.K.）、和風（Tropical Delight）和高峰（Summer Summit）等三種甘藍，以 MS 培養基進行固態培養，將花蕾分成七級（表2），每級四重複，每重複6個花藥。

表 2. 三種甘藍不同花蕾大小其花藥培養的癒合組織誘導率

Table 2. Percentage of callus formation from anther culture of different development stages of 3 cabbage varieties.

Size of flower buds (mm)	Summer Summit	Tropical Delight	Chu Chou (K.K.)	Total
1. grade 0～2	20.8(%)z	39.0(%)	0 (%)	19.1(%)
2. grade 2～3	0	19.2	31.8	16.9
3. grade 3～4	0	60.8	59.3	24.4
4. grade 4～5	0	23.0	0	7.5
5. grade 5～6	–y	4.3	8.3	8.1
6. grade 6～7	–	—	0	0
7. grade 7～8	–	—	10.0	3.7
Total	1.8	19.5	20.4	15.2

z anthers with 4 replication for pertreatment

y non test

(二)癒合組織誘導（callus induction）：培養基以 MS（Murashige and Skoog,1962）爲主，處理分固態和液態培養兩種，再依生長調節劑 BA(6-benzylamino purine)兩種濃度（0.5，1.0 ppm）和2,4-D（2,4-dichlorophenoxyacetic acid）四種濃度（0，0.5，1.0，2.0 ppm），搭配成不同組合，並以不含此二種生長調節劑之培養基爲對照，合計18種處理，每處理四重複，每重複六個花藥。洋菜濃度爲0.8%，蔗糖含量爲3%。

(三)癒合組織再分化誘導：由花藥所衍生的癒合組織，生長達0.5公分以上者，移至含有0.5 ppm BA 及不同 NAA（α-naphthalene acetic acid）濃度（0，0.5及1 ppm）的三種分化培養基，誘導再生（由藥柄處長出的則不繼代培養）。

(四)試管苗的移出：將再生之不定芽切下，扦插於含 MS 的發根培養基（以二份蛭石加一份珍珠石取代洋菜當介質），於發根後加強光照二至三週以健化植株；再將小植株移出試管，先假植於含有蛭石與珍珠石介質（相同於發根培養基）的塑膠籃內，蓋上塑膠膜以減少蒸散作用；並置於每日16小時光照、溫度在20～22°C之生長箱中，經一到二週後再移到溫室栽植。

結　果

和風、高峰和初秋三種甘藍，取不同大小之花蕾（圖1），先進行不同發育期之花藥培養，並同時觀察其花粉發育過程。結果顯示花蕾在3～4 mm大小時培養，可獲得最高之癒合組織生成率、0～2 mm 次之，4 mm 以上則較差（表2）。唯2 mm以下花蕾，因其花藥極幼小，操作困難易帶有花絲。因此往後取材均以2～4 mm 左右之花蕾爲主。經鏡檢得知此花粉發育期，大約是介於四分子期到晚單核期之間。

本試驗取甘藍和青花菜品種（系）各十一種（表1），進行不同的生長調節劑濃度、液態與固態培養，以瞭解基因型、培養基和生長調節劑對癒合組織再生之影響。結果顯示不同品種的癒合組織生成時期早晚大致一樣，在培養第一、二週即可看到花藥開裂；培養三、四週後，可發現由開裂處長出癒合組織（圖2）。青花菜在液態和固態培養下，雖其癒合組織平均生成率皆差不多，但品種間表現則差異頗大（表3）；固態培養基的生成率從0到12.2%，其中以 TVI6671 品種表現最好，最差是綠芳品種；液態則從0到10.6%，以 TVI6669 品種最好；而在固、液態二者中表現均不錯的是 TVI6669 和 TVI6723 兩品種。甘藍的癒合組織生成率除了 91-151 品系固態培養比液態培養較好外，其餘10種均以液態培養較佳，誘導癒合組織的生成率，平均比固態高出兩倍多（表3）。不同品

種（系）甘藍固態培養，癒合組織生成率從 0 到11.8%；其中以 91-087 和 91-116 品系表現爲佳，最差的是 91-118，91-130 和純系-3等三品系；液態培養則品種（系）間差異最大，從 0 到47.3%；其中生成率最好的也是 91-116 品系，大約每二個花藥就有一個長出癒合組織，91-130 品系則表現最差，在固態和液態培養下，花藥均很快的褐化逐漸變黑而死亡。唯液態培養的甘藍和青花菜，很多癒合組織是由花藥與藥柄接觸地方長出來的；且不論是青花菜或甘藍，由液態培養而來的癒合組織質地均較光滑且鬆散，經繼代培養後易褐化或祗進行癒合組織增生、其再生能力均較差（圖 3）。

表 3. 不同甘藍與青花菜品種在固態或液態培養基下之癒合組織生成率[z]

Table 3. The ratio of callus formation on liquid or solid culture medium in different cabbage and broccoli varieties.

Cabbage (%)			Broccoli (%)		
Variety	Solid	Liquid	Variety	Solid	Liquid
初秋	3.4	13.8	綠王	3.1	1.2
純系-3	0	0.5	綠芳	0	2.0
91-087	10	19.8	TVI6665	2.5	3.7
91-112	1.1	9.4	TVI6666	1.1	1.0
91-116	11.8	47.3	TVI6667	1.4	6.2
91-118	0	0.5	TVI6669	4.2	10.6
91-130	0	0	TVI6670	2.0	0
91-131	2.1	3.5	TVI6671	12.2	0
91-151	2.7	0.4	TVI6723	11.8	4.7
91-177	3.3	9.3	TVI6728	3.2	0.9
91-185	0.5	2.6	TVI6734	1.0	0
Average	3.07	8.95		2.99	2.88

[z] 9 treatments×6 anthers×4 replication

植物生長調節劑處理上，BA 的效果整體而言頗佳（表 5）。平均而言，甘藍和青花菜在含有 BA 濃度0.5 ppm 時，表現較 1 ppm 爲佳（表 4），在液態或固態的情形也一樣（表 5）。而各品種（系）的表現，除 91-131 甘藍和三種青花菜（TVI6666，TVI6667 與 TVI6734）等品種（系）外，也都有這種趨勢（表 5）。2，4-D 的處理效果，不同種（青花菜或甘藍）之間對2，4-D 的反應，會隨固態或液態的處理而有所不同（表 5）。固態培養的甘藍隨2，4-D 濃度上升，其生成率也隨之上升，平均以 2 ppm最佳；而青花菜的固態培養平均以 1 ppm爲最佳，0.5 ppm 次之。液態培養的甘藍除 0 ppm 外，剛好和固態培養的相反，2，4-D 濃度愈高其生成率反而愈低；而液態培養的青花菜，平均則以0.5 ppm 最高，2 ppm 次。但若依青花菜或甘藍的癒合組織生成率總平均值來比較，則表現頗爲一致；即在2，4-D 0 ppm 時癒合組織的生成率極低，0.5 ppm 最佳，但隨濃度提高而生成率有逐漸降低的趨勢。在所有的甘藍和青花菜品種（系）之間，對2，4-D 的反應（表 4）；是以 91-116 品系甘藍表現最好，0.5及 1 ppm 的2，4-D 處理，其生成率均可達50%左右；其次是初秋在0.5 ppm 濃度下，生成率達27.3%；而 91-087 品系在0.5，1 和 2 ppm 也均有很好的表現，平均都在20%以上。青花菜品種則除 TVI6670 和 TVI6723 兩品種表現較佳外，其餘均不理想。而兩種作物中，表現最差的則是甘藍 91-130 品系，均無癒合組織的生成。

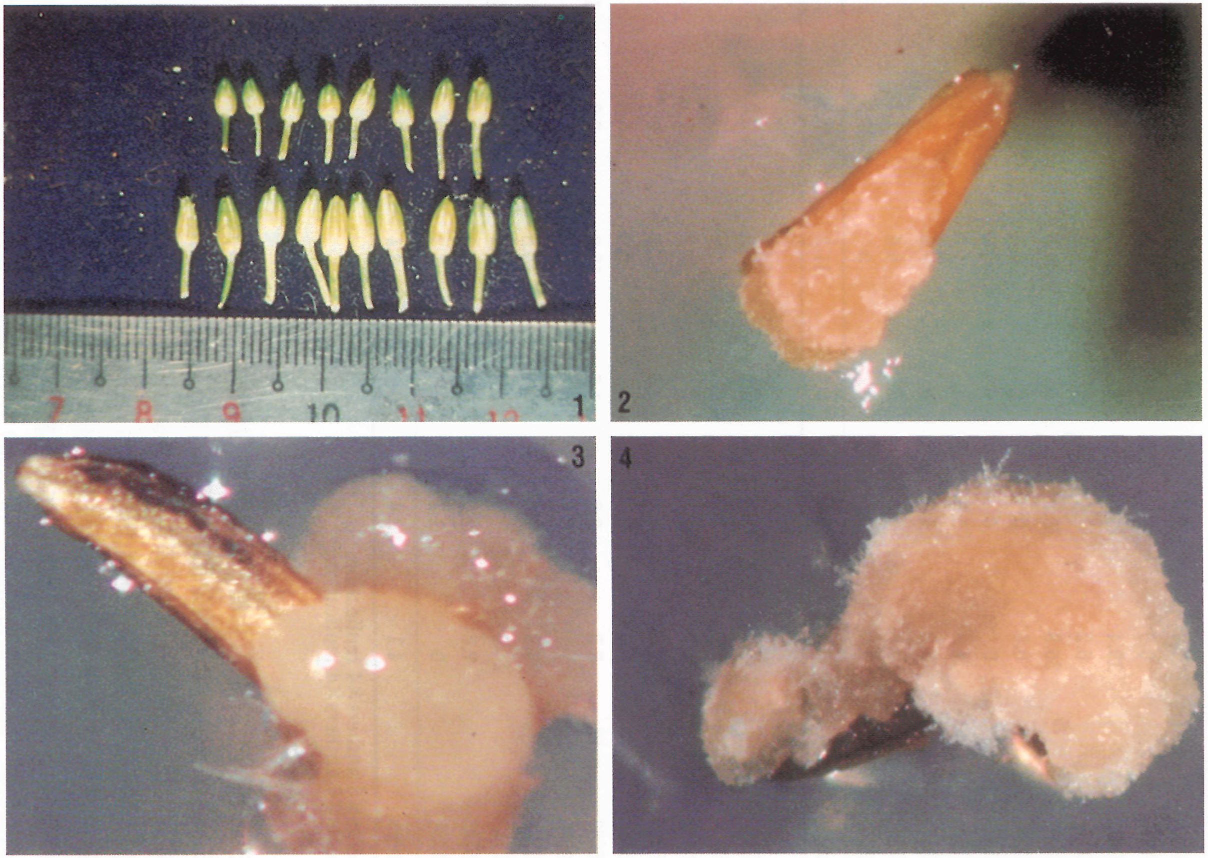


圖 1. 甘藍參試培養用之花藥大小，上排由左至右爲 2～4 mm
Fig. 1. Size of flower buds in anther culture of cabbage. Respectively, 2～4 mm from left to right on up line.

圖 2. 培養三～四週自開裂花藥長出癒合組織
Fig. 2. Callus derived from openning anther after 3～4 weeks in culture.

圖 3. 表面光滑，質地鬆散的癒合組織
Fig. 3. Smooth surface and friable texture of callus.

圖 4. 表面粗糙、質地緊密的癒合組織
Fig. 4. Rough surface and firm texture of calus.

表 4. 11種甘藍和11種青花菜花藥在不同 BA 與 2，4-D 濃度下的癒合組織生成率

Table 4. The of callus formation on different concentrations of BA and 2,4-D in 11 cabbage and 11 broccoli varieties.

variety	BA(ppm)^z		2，4-D (ppm)^y			
	0.5	1.0	0	0.5	1.0	2.0
cabbage (%)						
初秋	4.3	3.3	0	27.3	4.3	13.8
純系-3	0.6	0	0	1.2	0	0
91-087	19.4	13.8	0	22.9	20.0	24.7
91-112	6.2	1.1	0	1.1	2.2	3.2
91-116	36.9	30.8	0	49.5	53.9	31.9
91-118	0.5	0	0	0	1.2	0
91-130	0	0	0	0	0	0
91-131	1.3	5.5	0	1.3	7.1	2.7
91-151	2.2	1.2	0	7.8	0	0
91-177	9.9	6.1	0	4.4	1.1	7.2
91-185	3.0	0.5	0	0	5.3	1.3
Average	8.3	5.4	0.1	11.3	9.3	7.1
broccoli (%)						
綠王	4.7	0.3	2.5	3.0	3.0	0
綠芳	1.3	0.08	0	1.7	1.5	1.0
TVI6665	5.7	1.0	0	8.2	1.1	3.9
TVI6666	0.6	1.1	1.6	1.1	0	1.2
TVI6667	4.0	5.08	0	7.2	5.8	4.9
TVI6669	8.9	8.3	1.5	12.8	8.2	11.0
TVI6670	1.1	0.8	0	0	2.6	1.5
TVI6671	3.9	4.0	0	10.8	1.2	3.4
TVI6723	14.0	4.0	0	9.6	16.7	11.8
TVI6728	2.6	2.0	0	3.8	5.1	0
TVI6734	0	1.3	0	1.3	0	1.4
Average	4.1	2.4	0.6	5.1	3.9	3.1

z 2 treatments×6 anthers×4 replication
y 4 treatments×6 anthers×4 replication

將大小在0.5公分以上，表面粗糙、質地緊密的癒合組織（圖 4），平均分配的移至含有0.5 ppm BA 和三種不同 NAA 濃度的分化培養基中（表 6）；結果顯示不含 NAA 或含有0.5 ppm 均能誘導癒合組織分化，長出叢生狀綠色芽球體（圖 5），進而分化成含根莖葉的小植株（圖 6）；此再生之途徑是走間接器官（組織）發生（indirect organogenesis）的途徑。由一個花藥來的癒合組織，可陸續誘導出 5 ～20株不等的小植株。將小苗分株切下，移到不含生長調節劑的 MS 培養基中，並用二份蛭石加上一份珍珠石當介質取代洋菜（圖 7），並提高糖含量至 6 %，以促進發根。約一到二週即可長出新根，此時提高光照強度（約3,000 LUX 以上）以健化其生長，約再經半個月，即可移出試管。不用洗清根部，直接假植在蛭石加珍珠石的混合介質中，用塑膠籃上面覆以保鮮膜或塑膠膜，置於每天照光16小時，溫度20～22°C的小型生長箱中，經一周後掀去覆蓋膜並移至溫室中，81株外移材料中僅 1 棵因植株太小，根系不佳，在水份 太多下腐爛，其成活率高達98%以上(圖 8)。取部份植株的根尖，經初步的染色體觀察，證實有單倍體植株再生。

表 5. 不同 BA 與2，4-D 濃度在固態或液態培養基下甘藍和青花菜花藥之癒合組織生成率

Table 5. The average ratio of callus formation on different conce- ntrations of BA and 2,4-D with liquid and solid medium coniditon in 11 cabbage and 11 broccoli varieties.

Medium	BA(ppm)	2，4-D				Total	Percentag
		0	0.5	1.0	2.0(ppm)		(%)
	cabbage						
Solid	0(ck)	0(244)[z]	-[y]	—	—	0(244)	0
	0.5	0(237)	12(227)	15(245)	11(247)	38(956)	3.97
	1.0	1(242)	4(216)	5(233)	17(224)	27(915)	2.95
Average of solid(%)		0.13	3.16	4.18	5.94		3.07
Liquid	0(ck)	0(252)	—	—	—	0(252)	0
	0.5	0(252)	54(241)	49(254)	19(234)	122(981)	12.44
	1.0	0(249)	35(247)	21(243)	19(230)	75(969)	7.774
Average of liquid(%)		0	18.24	14.08	8.19		8.96
Average of cabbage(%)		0.07	11.28	9.23	7.06		6.07
	broccoil						
Solid	0(ck)	0(310)	—	—	—	0(310)	0
	0.5	7(304)	15(323)	23(325)	14(313)	59(1,265)	4.66
	1.0	0(301)	12(310)	8(298)	04(295)	24(1,204)	1.99
Average of solid(%)		0.77	4.27	4.98	2.96		2.99
Liquid	0(ck)	1(304)	—	—	—	1(304)	0.33
	0.5	3(301)	25(322)	10(311)	6(316)	44(1,250)	3.52
	1.0	0(313)	13(319)	8(336)	15(294)	36(1,262)	2.85
Average of liquid(%)		0.44	5.93	2.78	3.44		2.88
Average of cabbage(%)		0.60	5.10	3.86	3.20		2.93

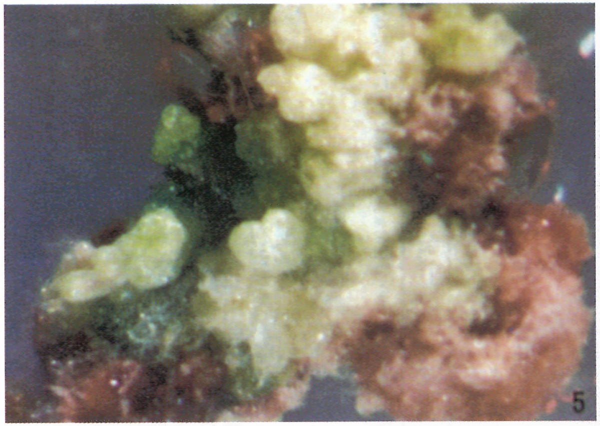
[z] In parenthesis represent the number of culture anthers, out parenthesis represent number of callusing anthers

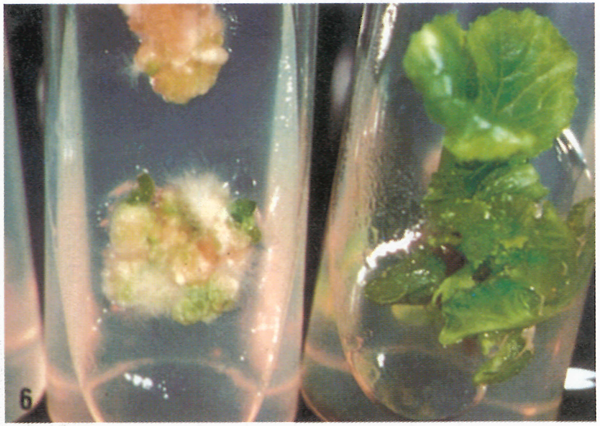
[y] non test

表 6. 2種甘藍和2種青花菜品種（系）花藥培養其誘導出植株之再生率

Table 6. The rate of plantlet formation from calli on 3 concentrations of NAA hormones in 2 cabbage and 2 broccoli varieties respectively.

variety	No. of anthers to cultured	No. of anthers forming calli	No. of anthers forming plantlets			Rate of anthers forming plantlets (%)
			0	0.5	1ppm(NAA)	
cabbage						
初秋	403	34	1	4	0	1.24
91-116	355	108	1	0	0	0.28
broccoli						
綠王	863	19	1	0	0	0.12
綠芳	838	8	0	1	0	0.12





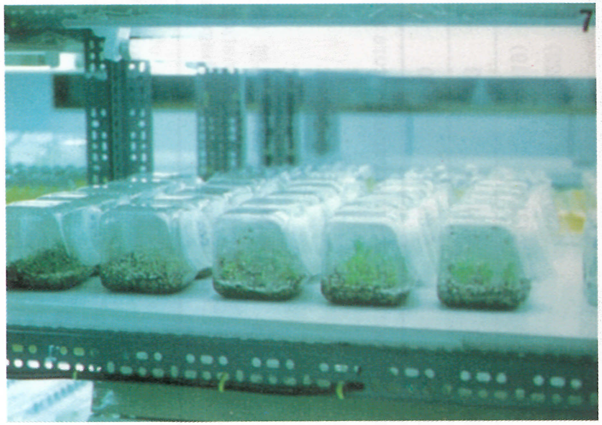


圖 5. 癒合組織長出之芽球
Fig. 5. Callus differentated into numerously organ-like structure.

圖 7. 微體繁殖與不定根誘導於無土介質
Fig. 7. Aseptical microcutting and root induction in soilless mediium.

圖 6. 由芽球繼續分化生長出小植株
Fig. 6. The adventitious buds development into plantlet.

圖 8. 小植株移出培養盒之成活情形
Fig. 8. Survival plantlets transplanted from tissue culture box.

討 論

許多報告指出花蕾的大小，影響花藥培養時體胚或癒合組織的形成[2,10]。Takahata 等人[24]認爲西洋油菜（*B. napus*）以2.5～3.5mm 最適合培養；Keller 等人[11]進行蕪菁（turnip rape, *B. campestris*）不同蕾期的培養，結果顯示在晚單核到早雙核期爲佳，Sato 等人[23]進行中國白菜的花藥培養，和 Quazi[22]的青花菜花藥培養，也均指出以 2～4 mm 大小的花蕾爲佳，此和本試驗結果一致，在 2～4 mm 大小的花蕾，即約四分子期以後到晚單核期間，具有較高的誘導率，但和何[2]的結果則相反，這可能是品種上的差異所致[21]。Keller 和 Armstrong[14]及 Arnison 等人[6]的報告顯示，高溫處理等變溫方式，有助於花藥培養時體胚或癒合組織的形成。因此本試驗採用高溫37°C暗處理一天，其效果比均無癒合組織長出的對照組（資料未發表）爲佳。

甘藍和青花菜參試品種（系）各11種，其品種間差異很大，Biddington 和 Robinson[7]及 Keller 和 Armstrong[14]均認爲甘藍類爲異交作物，具有自交不親和性，因此其各品種的基因型，甚至株間均有不同；這也許可以解釋在甘藍品系中 91-131，91-151，和 91-177三種雖同一雜交品系後代，但癒合組織生成率差異很大的原因（表 3，4）。根據 Phippen 和 Ockendon[21]指出花椰菜進行培養時，其體胚生成率比抱子甘藍差的原因是因爲花椰菜經春化處理後，其抽苔開花很快，盛花期短，花蕾間由於養分競爭劇烈，造成花藥培養的效率低。根據田間觀察，盛花期短之現象在青花菜更明顯（約 3～5 天），整個花蕾約有數百朵花，均於短時間內同時開放。而本試驗結果顯示，青花菜培養的癒合組織誤導率均比甘藍差，這可能是其原因之一。

Lichter 等人[17]和 Keller 等人[14]指出西洋油菜（*B. napus*）等一些蕓苔屬作物液態培養比固態好，青花菜[6]或其它作物如水稻[25]也以液態培養較佳；但 Phippen 和Ockendon[21]的結果則是以固態較好。Dunwell[8]則認爲此二種型態培養難以比較，因爲液態培養時，有些花藥會沉到底部，會因缺氧而無法長成體胚或癒合組織，因此比較時要考慮這些因素。本次試驗很少花藥沉底的情形，結果顯示癒合組織的誘導普遍以液態較佳。

一般花藥培養的 auxin 類，初期由花藥誘導體胚或癒合組織均以2，4-D 爲主[6,11]；也有的用 NAA，並認爲比 IAA 好[10]。本試驗2，4-D 的效果和 Quazi[22]或 Kameya 和 Hinata[10]等研究相似，以0.5～1 ppm 效果較好，太低或太高的濃度都不適合。在 cytokinin 方面，Quazi[22]的結果顯示 BAP（BA）濃度增加，其癒合組織生成率增加。本試驗顯示有些品種（系）如甘藍的 91-131 品系和青花菜的 TVI666，TVI6667 和 TVI6734 品系也有此現象。Keller 等人[11]在早期（1975）之實驗中，培養基上不加 BA；但到1983年代[14]以後的試驗，均加以 1 ppm BA 以提高[15]。雖然本試驗品種間差異很大，大致上還是 BA 0.5 ppm 的效果較好。

有些癒合組織生成率高的品系（如 91-116 品系），其繼代培養再生效果反而差；往往一經繼代培養便很快的褐化或玻璃質化（vitrification），此一現象值得繼續探討。另嚐試改用其它生長調節劑或塡加 Ag NO_3，citric acid 或 ascorbic acid 之類的藥劑，以某求改善褐化及分化能力偏低（表 6）的缺點，這將是下一步努力的重要之一。

表 6 所列的初秋甘藍及綠、王綠芳青花菜，爲本省之主要栽培品種，而 91-116 爲本所雜交之抗黑腐病之後代。其再生率雖然偏低，然統計祗針對經花藥培養後能獲得植株再生的花藥數；然而由一個花藥培養而來的癒合組織，均可獲得 5～20株陸續分化的小植株，因此實際獲得的植株數目遠大於此（目前有100餘株），且仍在繼續分化並長出小植株。植株採根尖進行鏡檢，以調查染色體的倍數性初步已有單倍體植株，除繼續進行根尖鏡檢外並進行染色體的倍加，以育成同質二倍體，供品種改良之用，將爲未來主要的工作。

根據試驗觀察結果得知，經由間接器官（組織）發生的植株，其所長的根，其根系大多是經由莖

基部癒合組織再長出，直接由莖基部長出很少。在傳統洋菜培養基下培養，當外移時均需清洗根部附著的洋菜，以防止雜菌汚染發黴，因此在清洗時往往會使植株莖葉受傷（因組織培養苗之植株細小又脆弱），且易將莖基部癒合組織弄掉損及根系；況且根長在癒合組織上對作物往後生長亦有不利之影響。因此若先將培養的洋菜介質，換成試管外育苗栽培用的介質；並利用扦插莖段的方式，讓莖基部重新長根後，再直接由試管中移到相同的介質上，同時以淸水澆灌，不必經過清洗的階段，結果顯示此法之成活率極高。其可能之原因，據調查利用這種方法有幾個特點：

一、莖基部經暗處理（蛭石和珍珠石不透光）白化後，較易促進根的生長。

二、介質鬆而不散（根系包圍），易移植，和外界介質相同適應性高。

三、移植時根系完整（類似穴盤育苗原理），可提高移植成活率。

四、不需清洗，可減少損傷腐爛及洋菜所造成的雜菌汚染。

五、高濃度的糖份、鹽類及有機質等培養液，可直接被稀釋，不會造成雜菌滋生。

可能由於這些有利的條件；使移植成活率高達98%（1僅株腐爛死亡）；此法應用於其他十字花科作物如千寶菜及小白菜的移植，存活率也高達95%以上（資料尚未發表）。因此若能利用此種方法，將可大爲提高蕓苔屬植物組織培養苗移植時的成活率。

初步染色體檢定，證實有單倍體植株再生，將進一步加強所有植株的染色體檢定；而單倍體植株的大量繁殖及染色體倍加的研究，是未來工作的重點。

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Anther Culture of Cabbage (*Brassica oleracea* L. var. *capitata*) and Broccoli (*B. oleracea* L. var. *italica*)

I. Varieties, Developmental Stages and Cultural Medium Relation with Regeneration[1]

You-Ming Chang, Pan-Chi Liou and Chi-Hsiung Hsiao[2]

Summary

Cabbage (*Brassica oleracea* L. var. *capitata*) and broccoli (*B. oleracea* L. var. *italica*) anthers containing microspores between tetrad and late uninucleate stages (flower bud is about 2 to 4 mm in length) were successfully cultured on MS medium supplemented with 2, 4-D and BA for callus induction. Among 11 cabbage varieties, the callus formation was much improved in liquid medium instead of agar medium, and characterized with smooth and friable texture. The callus formation rates were ranged from 0 to 47.3% varied with varieties. Similar result was also observed from 11 varieties of broccoli. Higher frequencies of callus formation were derived from anther cultured on MS medium containing 0.5 ppm BA and 0.5~1 ppm 2,4-D. The regeneration capability of anther-derived callus was tested by 0.5 ppm BA with 3 levels (0, 0.5 and 1.0ppm) of NAA. Shoot regenerated through indirect organogenesis were observed. For root induction, plantlet shoots were aseptically subcultured by cutting on vermiculite mixed with perlite, and this also gave a hardening treatment. Up to 98% of the plantlets were survived after transplanting using this method. Some haploid plants were detected by the chromosome assay of root tip tissue.

Key Words : Broccoli, Cabbage, Anther culture, Haploid.

1. Contribution No. 1829 from Taiwan Agricultural Research Institute.
2. Respectively, Assistant Horticulturist, Associate Horticulturist, and Senior Horticulturist and Head of Department of Horticulture, TARI, Wufeng, Taichung, Taiwan, ROC.

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不整地玉米膝高期淹施^{15}N—尿素後氮素之追蹤[1]

連　深[2]　王鐘和[2]　李瑞成[3]

摘要：本試驗前後於學甲兩處及霧峰一處不整地秋作玉米田進行。基肥於播種時施用　N、P_2O_5、K_2O 各70，105及70 kg/ha。試驗方法係於玉米膝高期設置內框和外框，以三種水量（5mm，50mm 及100mm）淹施尿素（以N計70 kg/ha）；內框內施用 5 atom％的^{15}N標識尿素，而內框與外框所圍部分則施用一般尿素，並於尿素淹施後的特定時日追蹤內框所施標識氮素在土壤中之動態及玉米之吸收。

試驗結果示氮肥的吸收率隨淹施的水量而異，並有顯著土壤間差異；滲透性較好的學甲土壤以 50mm 爲宜，而滲透性較差的霧峰土壤則以 5mm 爲宜。霧峰土壤淹施 50mm 以上水量則氮肥的吸收率顯著降低，而學甲土壤則以淹施 50～100mm 者氮肥吸收率較高。惟學甲土壤如淹施100mm 水量卻有氮肥滲濾至70公分以下層跡象，有污染地下水虞。

以上土壤間差異可能與土壤之 pH 值及淹施尿素後土壤水分含量之差異所致氨揮散和脫氮等差異有關，文中曾詳加討論。

關鍵詞：^{15}N、尿素、淹施、玉米、氮素吸收。

前報[4]以^{15}N標識的硫酸銨追蹤秋作玉米各生育期所施氮肥的吸收率，已知玉米不同生育期及耕作方法下所施氮肥之吸收率差異很大；基肥之吸收率最低，僅10～28％。膝高期追肥的吸收率最高，達44～58％，其中有整地之下所施追肥因有培土，吸收率較高，而不整地下所施肥料則因施肥後不培土，吸收率較低。

故膝高期乃玉米施肥之適當時期。膝高期施肥若以尿素隨適當的水量淹施（或於施肥後立即以適量的水淹灌），將使肥料滲入適當的土層中，可避免在土壤表面之揮散損失，亦有利於作物吸收；如有適當設施配合，且可節省施肥勞力。此種尿素之淹施在不整地栽培之下尤可適用，因在該栽培制度下所施氮肥無法培土，較易損失。此種尿素之淹施在玉米抽穗期亦可適用；因該生育期玉米植株已甚茂盛，人工追肥不易，施肥後亦無法培土易導致氮肥之揮散損失。許多試驗結果亦證實該生育期之追施氮肥（穗肥）有效，且有必要[2,3,5,8,9]。

但淹施尿素時，如水量過多將使肥料滲入過深的土層，不單不被作物吸收，亦會污染地下水。是故淹施之適當水量如何？土壤間有何差異？等，亟需加以探討。有關肥料的淹施，本省在甘蔗方面已有若干研究[6,7]。

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1. 臺灣省農業試驗所　研究報告第 1830 號。
2. 臺灣省農業試驗所研究員、助理研究員。臺灣省　臺中縣　霧峰鄉。
3. 行政院原子能委員會　核能研究所副研究員。臺灣省　桃園縣　龍潭鄉。

材料與方法

一、試驗地點之土壤性質及試驗期間

本試驗於1991年秋作在霧峰（省農試所農場）及學甲（頂州）各一處進行，1992年秋作則在學甲（大彎）一處進行。試驗地點之一般土壤性質如表 1，剖面各層土壤之水分特性及1/3巴氣壓下之空氣孔隙率如表 2 及 3。

表 1. 各試驗地一般土壤性質

Table 1. General properties of soils at each experimental location.

Location	Depth (cm)	Parent material	Soil series	Texture	Permeability	pH	Organic matter(%)
霧峰（農試所）	0～15	粘板岩、砂頁岩	七塊厝系	SiL	mod. low	5.3	1.9
Wu-feng (TARI)	15～30	混合新沖積		SiL	-low	6.4	1.3
學甲（頂州）	0～15	石灰性砂頁岩	將軍系	SiL	high	8.5	1.0
Hsueh-chia (Ding-chow)	15～30	沖積		SiL		8.6	0.7
學甲（大彎）	0～15	石灰性砂頁岩	岸內系	SiL	mod. high	6.1	2.4
Hsueh-chia (Dah-wan)	15～30	沖積		SiL		7.2	1.4

表 2. 霧峰試驗地土壤剖面各層之土壤水分張力特性及田間容水量（1/3巴）下之空氣孔隙率

Table 2. Characteristic of moisture tension and air-filled porosity at field capacity (1/3 bar) at each depth in the profile of Wu-feng soil.

Depth of soil (cm)	Moisture content (v/v,%)			Air-filled porosity(v/v,%) at 1/3 bar
	1/100 bar	1/20 bar	1/3 bar	
0～15	43.9	38.0	33.7	10.2
15～30	39.1	35.0	32.1	7.0
30～45	41.2	36.3	31.4	9.8

表 3. 學甲（大彎）試驗地土壤剖面各層之土壤水分張力特性及田間容水量（1/3巴）下之空氣孔隙率

Table 3. Characteristics of moisture tension and air-filled porosity at field capacity (1/3 bar) at each depth in the profile of Hsueh-chia soil.

Depth of soil (cm)	Moisture content (v/v,%)			Air-filled porosity(v/v,%) at 1/3 bar
	1/100 bar	1/20 bar	1/3 bar	
0～ 18	57.8	48.5	37.8	20.0
18～ 26	44.3	39.6	36.4	7.9
26～ 47	44.3	41.1	37.1	7.2
47～ 81	48.6	43.8	38.2	10.4
81～113	49.7	45.3	42.4	7.3
113～150	52.9	49.2	45.9	7.0

二、秋作玉米之栽培方式及試驗處理

各試驗地之玉米栽培均以不整地方式進行；秋作玉米前均有夏作田菁，並於田菁高達1.5～2.0m時以「圓盤切割式施肥播種機」施用基肥（N、P_2O_5、K_2O 各70，105，70kg/ha 以臺肥複合肥料39號供之），同時播種玉米，而被壓倒的田菁則為玉米田之敷蓋[1]。

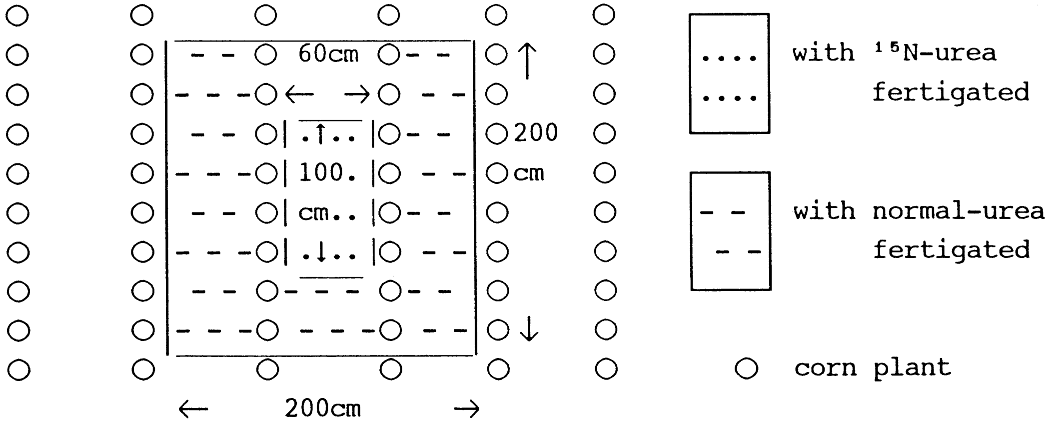
㈠試驗處理：

以不整地方式栽培秋作玉米，而於玉米膝高期設置下列三種不同水量（5，50及100mm，但頂州一地以5mm，25mm 及50mm）之尿素淹施（fertigation）處理。尿素用量一律以氮素計70kg/ha，溶於各處理水量後淹灌施用。

各處理區實施淹灌處理時均設有內框與外框；內框淹灌^{15}N 標識之尿素，而內框與外框之間則淹灌一般尿素，二者之淹灌水深（即水壓）相同，可避免內框所淹灌之標識尿素易滲入其框外，詳細如下節㈡之1。各處理區之間均有5公尺以上距離，以免相互間有任何因不同水量淹灌所導致之影響。各處理區均設2重複，以完全逢機方式排列。

㈡處理方法：

1. 內、外框之設置：以鐵製內框（60cm×100cm）之短邊（60cm）靠於兩行玉米（行距70cm）之內側，長邊靠於各行4株（株距25cm）之內側（頂州試地則為各行5株），放置後將框之基部打入土中約3cm，使之穩固（因框高為20cm，故設置後之框高約17cm）。再將外框（200cm×200cm）設置（亦是鐵製）使內框的位置處在其正中央，詳細如圖。外框設置方法如同內框。



2. 膝高期尿素肥料施用方法：尿素施用量一律以N計，70kg/ha，均溶於各處理及內、外各框面積（外框部分需扣除內框面積）所決定之用水量後才淹施於各個框內。內框使用^{15}N（5atom%）標識之尿素，而外框內（扣去內框部分）則使用一般之尿素。外框之外之非試驗區亦同時追施尿素，施用量與試驗區相同，惟不另予灌水。

㈢調查測定項目

1. 學甲（大彎）試驗地於尿素肥料淹施前及淹施後2及98天（玉米收穫時），採取各處理區內框內土壤，自土表0～5，5～10，10～15，15～20，20～30，30～40，40～50，50～60，60～70，70～80cm 各層樣本。樣本之採取係以內徑5.7公分之土鑽為之。每次樣本之採取係自內框（長方形）較長的各端各採一孔，故每個內框可採得各個土層之土壤本兩個。採土後遺留之孔則以鄰田採取相對土層之土壤填充，以免影響其餘內框試區之完整性，可供繼續往後之採樣。所採得之土壤樣本均立即供水分和銨態氮、硝酸態氮之測定，並經風乾後供全氮和^{15}N 富含量之測定。

霧峰試驗地亦於淹施前及淹施後(但於1，2及8天)，以同法採取土壤，供水分之測定。

2. 於玉米成熟期收穫內框兩側之玉米各 2 株（即各側 4 株中去除邊緣各 2 株）合計 4 株（頂州試地則收穫內框兩側之玉米各 3 株，合計得 6 株），烘乾後分爲子實和藁（包括根、莖、葉、葉鞘、穗軸及穗苞葉）各部分，稱重，磨粉後並測氮素含量及 ^{15}N 富含量，以求得各種處理下之玉米全株 ^{15}N 吸收量，再除以相當於該 4 株（頂州試地爲 6 株）所施予之尿素—^{15}N 量，即可求得尿素肥料之吸收率。

結果與討論

一、玉米生育狀況及膝高期所淹施尿素之吸收利用情形

(一)玉米生育狀況

本試驗以三種水量淹施^{15}N—尿素（以N計，70 kg/ha）於膝高期玉米田，結果玉米收穫期各區之收量、氮素含量及吸收量如表 4 ，5 及 6 。

由表 4 可見霧峰試地各處理區之子實收量和氮素吸收量均以 5mm 區爲最高（子實收量408 g/4 plant），依次爲50及100mm 區，100mm 區之子實收量335g/4 plant 最低，惟二者之差異並不顯著（子實收量相當於5.5～6.7t/ha，以含水量13.5%計。以下亦同）。表 5 學甲頂州試地則示各處理區子實收量和氮素吸收量均以50mm 區爲最高，5 及 25mm 區較低（子實收量490～581 g/6plant，相當於5.4～6.4 t/ha）。表 6 學甲大彎亦示各處理收量和氮素含量均以 50mm 區和100mm 區爲最高，二者相差無幾，而 5mm 則爲最低（子實收量362～418 g/4 plant，相當於5.9～6.9t/ha）。

表 4. 霧峰農試所各處理區玉米收量^{z}及氮素含量（1991秋作）

Table 4. Yields and contents of nitrogen at harvest of the maize plants fertigated with different depths of water at knee-high stage (Wu-feng, TARI, 1991 fall crop)

Treatment (mm,water)	g./4 Plants		Harvest Index	% N		N (g./4 plants)		
	Grain	Stover		Grain	Stover	Grain	Stover	Total
5	408a	568a	0.42	1.34	0.85	5.45	4.86	10.31
50	370a	561a	0.40	1.38	0.80	5.13	4.49	9.62
100	335a	499a	0.40	1.38	0.80	5.08	4.01	9.09

^{z} Values in columns sharing a common letter are not significantly different (p=0.05) by Duncan's multiple range test.

表 5. 學甲頂州各處理區玉米收量^{z}及氮素含量（1991秋作）

Table 5. Yields and contents of nitrogen at harvest of the maize plants fertigated with different depths of water at knee-high stage (Hsueh-chia, Ding-dhow, 1991 fall crop)

Treatment (mm,water)	g./6 Plants		Harvest Index	% N		N (g./6 plants)		
	Grain	Stover		Grain	Stover	Grain	Stover	Total
5	516a	791a	0.40	1.63	0.83	8.40	6.62	15.02
25	490a	834a	0.37	1.63	0.90	7.99	7.53	15.52
50	581a	951a	0.38	1.60	0.83	9.30	7.87	17.17

^{z} The same as in Table 4.

表 6. 學甲大彎各處理區玉米收量^{z}及氮素含量（1992秋作）

Table 6. Yields and contents of nitrogen at harvest of the maize plants fertigated with different depths of water at knee-high stage (Hsueh-chia, Dah-wan, 1992 fall crop)

Treatment (mm,water)	g./4 Plants		Harvest Index	% N		N (g./4 plants)		
	Grain	Stover		Grain	Stover	Grain	Stover	Total
5	362a	527a	0.41	1.70	0.94	6.12	4.93	11.0
50	398a	592a	0.40	1.60	1.01	6.35	5.94	12.3
100	418a	662a	0.39	1.71	0.94	7.14	6.21	13.4

z The same as in Table 4.

以上結果概示霧峰試地之淹施水量以 5mm 區較優；50及 100mm 區較差，尤以 100mm 爲最。反之，學甲二試驗地則以 50～100mm 區較優，5mm 區最差。惟以上差異均未達顯著標準，乃因試區面積小，試驗植株只有 4～6 株，變異大之故。此種試區小之缺點卻可以以同位素追蹤技術提高試驗靈敏度獲得補救；此亦即本研究以標識之^{15}N 尿素探討有關問題之緣由。

各試驗地玉米膝高期以不同量水淹施之尿素標識氮素在收穫期之分布及吸收率如表 7，8 及 9。

㈡標識尿素之吸收率

由表 7，8 及 9 可見氮肥的吸收率隨淹施的水量有顯著差異；霧峰試地以 5mm 區最高，50 mm 區則顯著降低氮肥的吸收率（表 7）。學甲兩處試驗地均以 5mm 區之吸收率爲最低，並以 50mm 區或 100mm 區爲最高（表 8 及 9）。

表 7. 霧峰農試所各處理區膝高期所施尿素—^{15}N之玉米吸收利用情形^{z}（1991秋作）

Table 7. Percentage uptake by maize at harvest of the tagged urea-N fertigated with different depths of water at knee-high stage (Wu-feng, TARI, 1991 fall crop)

Treatment (mm,water)	%^{15}N excess(x10^{-3})		^{15}N uptake(mg/4 plants)			%uptake of the urea treated
	Grain	Stover	Grain	Stover	Total	
5	416a	392a	22.1a	18.5a	40.6a	35.2a
50	313a	299a	15.9ab	13.4ab	29.3ab	25.4ab
100	213a	191a	9.7b	7.6b	17.3b	15.0b

z Values in columns sharing a common letter are not significantly different (p=0.05) by Duncan's multiple range test.

表 8. 學甲頂州各處理區膝高期所施尿素—^{15}N之玉米吸收利用情形^{z}（1991秋作）

Table 8. Percentage uptake by maize at harvest of the tagged urea-N fertigated with different depths of water at knee-high stage (Hsueh-chia, Ding-chow, 1991 fall crop)

Treatment (mm,water)	%^{15}N excess(x10^{-3})		^{15}N uptake(mg/6 plants)			%uptake of the urea treated
	Grain	Stover	Grain	Stover	Total	
5	106b	76b	8.8b	5.0b	13.8b	7.4b
25	401a	410a	32.2a	30.6a	62.8a	33.7a
50	423a	399a	39.3a	31.4a	70.7a	38.0a

z The same as in Table 7.

表 9. 學甲大彎各處理區膝高期所施尿素—^{15}N之玉米吸收利用情形[z]（1992秋作）

Table 9. Percentage uptake by maize at harvest of the tagged urea-N fertigated with different depths of water at knee-high stage (Hsueh-chia, Dah-wan, 1992 fall crop)

Treatment (mm,water)	%^{15}N excess(x10^{-3})		^{15}N uptake(mg/4 plants)			%uptake of the urea treated
	Grain	Stover	Grain	Stover	Total	
5	74b	68b	4.8b	3.3b	8.1b	7.0b
50	334a	326a	21.1a	19.2a	40.3a	34.8a
100	324a	342a	23.1a	21.3a	44.3a	38.4a

[z] The same as in Table 7.

霧峰一地淹水量增加時，所施氮素之吸收率降低可能與該地土壤滲透率較差，淹水後表層（0～20cm）土壤含水量有一段時間偏高（對照表10及表2可見其淹灌 50mm 以上者，水分張力在淹後8天仍概低於1/20bar），通氣性較差，以致施肥後之脫氮作用較盛等有關，惟確切數據仍待探求。學甲兩試驗地5mm區之肥料氮素吸收率最低預料係與此等土壤之 pH 值較高有關；在淹水量很少的情形下，所施尿素均留在土壤表層，一方面增加氨揮散，另一方面亦因氮素未能滲入根群旺盛的土層，均會導致其吸收率降低。

學甲土壤因滲透性較佳，即使淹施 50～100mm 亦未導致土壤之含水量過高（對照表11及表3可見其0～60公分土層之水分張力於淹施後2天時均概已提高至1/3bar 以上），預料不致有顯著的氮損失，故無論是 50mm 或 100mm 區，肥料氮素的吸收率均高。惟從土壤剖面中標識氮素之分布觀之，100mm 區卻有肥料氮素滲入70cm以下土層跡象。此等滲入深層之氮素，由表9看來，顯然不致於對玉米之吸收利用有何顯著的不良影響，惟其是否有污染地下水嫌，擬於下節討論。

二、膝高期淹施尿素在土壤中之入滲轉化情形

(一)霧峰及學甲（大彎）各試驗地玉米膝高期尿素淹施前及後各區土壤剖面之水分分布情形如表10及11。

表 10. 霧峰農試驗地以不同量水淹灌後1，2及8天時土壤剖面各層水分含量（v/v，%）（1991秋作，膝高期）

Table 10. Volumetric moisture content at each depth in the profile of Wu-feng soil at 1，2 and 8 days after flood fertigation. (1991 fall crop, knee-high stage)

Depths of soil(cm)	Before fertigation	5 mm, flood fertigation			50 mm, flood fertigation			100 mm, flood fertigation		
		1 day	2 days	8 days	1 day	2 days	8 days	1 day	2 days	8 days
0～ 5	37.0	36.8	32.2	32.6	46.7	45.9	37.2	49.5	47.0	37.2
5～10	34.0	34.0	32.6	31.9	38.9	41.7	35.4	40.5	38.4	35.0
10～20	38.3	37.2	36.6	33.0	39.4	40.6	40.0	42.9	41.8	40.0
20～30	28.4	28.4	27.0	24.2	33.2	33.0	29.4	33.1	32.7	31.6
30～40	26.9	28.4	26.9	26.4	31.9	30.4	27.9	29.3	30.0	27.9
40～50	26.9	28.7	29.1	27.9	32.0	28.8	30.0	32.9	30.7	29.7
50～60	27.5	28.3	28.3	25.2	28.3	30.3	31.2	33.0	31.1	31.4
60～70	27.4	26.8	—	27.6	28.4	31.2	30.3	29.0	—	30.2
70～80	26.3	26.8	29.0	24.3	28.5	—	29.9	25.4	26.5	—

表 11. 學甲(大彎)試驗地以不同量水淹灌後 2 天時，土壤剖面各層之水份含量(v/v，%)(1992秋作，膝高期)
Table 11. Volumetric moisture content at each depth in the profile of Hsueh-chia soil at 2 days after flood fertigation. (1992 fall crop, knee-high stage)

Depth of soil (cm)	Before fertigation	flood fertigation		
		5 mm	50 mm	100 mm
0～ 5	18.0	23.9	40.3	41.3
5～10	24.3	26.9	35.4	35.9
10～15	25.2	28.3	34.3	35.7
15～20	28.6	31.1	35.4	34.5
20～30	29.0	30.5	34.6	34.7
30～40	30.3	30.0	34.1	32.7
40～50	32.0	—	34.0	34.3
50～60	35.3	—	36.8	38.0
60～70	36.2	—	—	37.9
70～80	36.8	—	—	37.0

學甲試驗地各區淹灌後 2 天時，土壤剖面各層水分含量之增加情形另示於圖 1 。

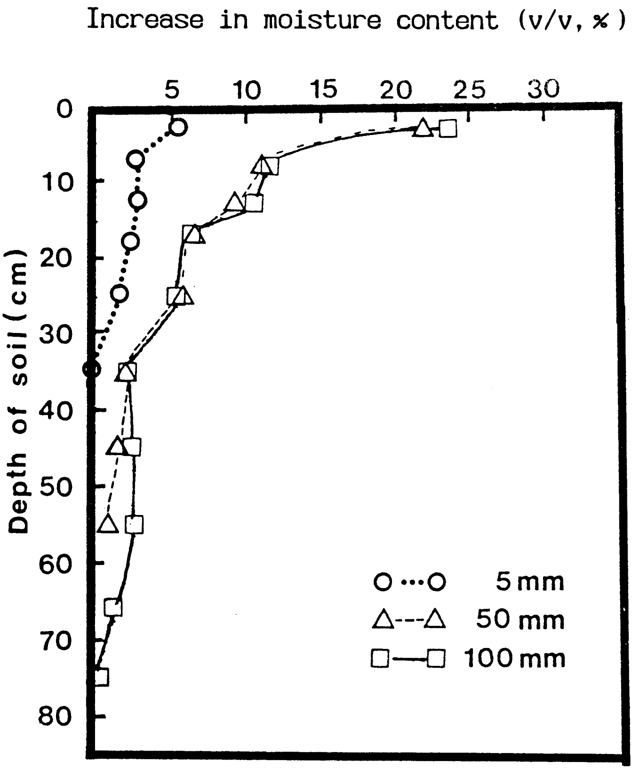


圖 1. 學甲試驗地淹施後 2 天時土壤剖面各層水分含量（容量%）之增加（學甲大彎，1992秋作，膝高期）
Fig 1. Increase in volumetric moisture content at each depth in the profile of Haueh-chia soil at 2 days after flood fertigation. (Hsueh-chia, Dah-wan, 1992 fall crop, knee-high stage)

㈡學甲試驗地淹施尿素後 2 天時各層土壤無機態氮和標識氮含量之分布。

學甲試驗地膝高期尿素淹施前及淹施後 2 天各層土壤中無機態氮濃度之變動情形如表12。

由表12可見尿素淹施後 2 天，除 100mm 區 0-5cm 層外，各層土壤均有無機態氮濃度的顯著增加；不同土層所增加的無機態氮濃度，依淹水量之多寡而有顯著差異。各層土壤無機態氮之80～90%均爲硝酸態（詳細數據因篇幅關係從略），餘者則爲銨態。可見學甲試地不單尿素之銨轉化迅速，其銨之硝酸化亦甚迅速。

表 12. 學甲試驗地淹施前及後 2 天土壤剖面各層之無機態氮素含量 (mg/kg)[z]（學甲大彎，1992秋作膝高期）

Table 12. Concentration (mg/kg)[z] of inorganic N at each depth in the profile of Hsueh-chia soil before and at two days after fertigation
(Hsueh-chia, Dah-wan, 1992 fall crop, knee-high stage)

Depth (cm) of soil \ Date of sampling Treatment (mm)	Before fertigation[y]	2 days after fertigation[x]			
		5	50	50*	100
0～ 5	61.2(24.1)	109.7(21.0)	68.4(7.2)	92.6(34.5)	36.1(3.3)
5～10	24.0 (3.6)	40.6 (2.9)	43.8(7.3)	65.7 (8.1)	32.9(1.1)
10～15	18.3 (0.3)	30.0 (4.8)	32.1(4.2)	53.5 (6.2)	30.7(0.8)
15～20	18.9 (2.2)	22.9 (4.1)	29.4(3.1)	41.4 (8.6)	26.4(0.6)
20～30	15.5 (1.0)	16.1 (0.9)	20.3(4.2)	33.3 (4.1)	16.6(2.5)
30～40	15.5 (7.9)	15.5 (8.8)	19.3(1.1)	22.9 (4.2)	18.7(0.9)
40～50	11.0 (2.2)		18.5(6.8)	22.0 (0.9)	12.2(4.1)
50～60	10.2 (1.4)		21.7(0.3)	17.3 (4.1)	19.1(2.2)
60～70	11.9 (1.0)				15.8(6.2)
70～80	20.9 (0.9)				18.2(2.0)

z Mean of two replications with standard error in parenthesis.
y 538 kg/ha of compound fertilizer, corresponding to 70，105and 70 kg/ha of N, P_2O_5 and K_2O, respectively was side-dressed to all the treatment (plots) as basal at sowing.
x 70 kg/ha of urea-N was fertigated with 5，50 and 100 mm depth of water, respectively to each treatment (plot) at knee-high stage as top-dressing. The rate of N was doubled in the treatment (plot) 50*.

表12更示以 5mm 水淹施尿素的試區，施肥後 2 日，在表土 0 ～15cm 層，尤其是其中之 0 ～15cm 層，有顯著的無機氮濃度增加，但15cm 以下各層則否，顯示所施肥料僅影響 0 ～15cm 以上表層。以100mm 水淹施者則其無機態氮濃度在 0 ～5cm 層反而減少，而在 5 ～70cm 層則有顯著的增加。顯示所施肥料已滲至 60～70cm 深層。至於 50mm 區則在 0 ～60cm 各層均見施肥所致無機態氮之增加，但未如 100mm 區因淹灌水量多，連表層原有的無機態氮亦淋洗至下層的現象。

對照圖 1 及表12可見尿素在學甲土壤中隨著淹施水量的增加往下之入滲甚爲明顯；當水量增至 100mm 時甚至將 0 ～5cm 表層原有之無機態氮淋下，並且其尿素氮亦已迅速轉化爲銨態並由銨態迅速更轉化爲硝酸態氮，而有滲下 60～80cm 層跡象。這種跡象由^{15}N—尿素淹施後 2 天各土層 ^{15}N 富含量之分布更易明暸（圖 2 ）此種滲入深層之氮素，依據表 9 玉米之 ^{15}N 吸收利用率在 50mm 區與 100mm 區並無顯著差異之事實看來，顯然仍可爲玉米吸收利用，惟另由本試驗地地下水位有時可昇至80～90cm事實看來，則其滲濾至 70～80cm 土層之硝酸態氮素亦不無污染地下水虞。

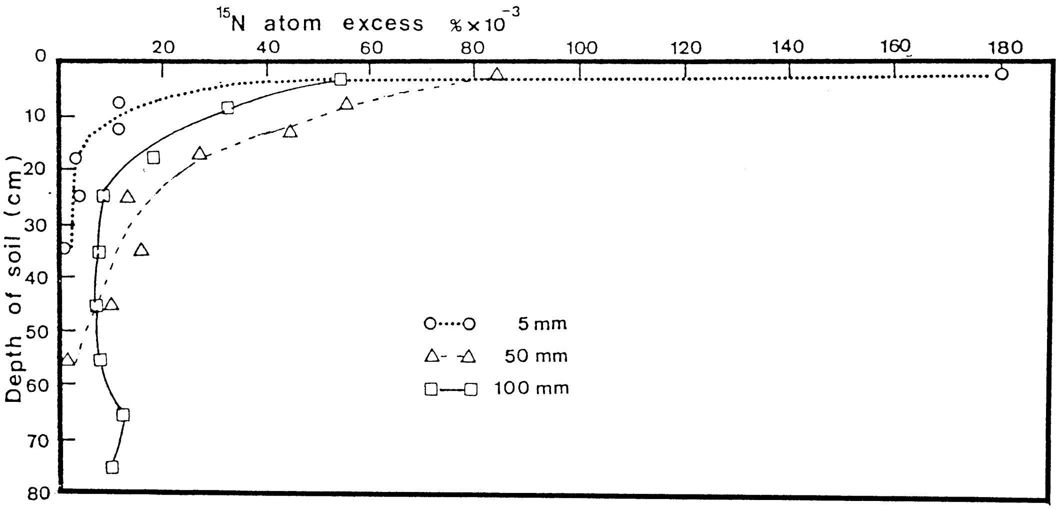


圖 2. 學甲試驗地以不同量水淹施^{15}N－尿素後 2 天，土壤剖面各層中標識氮素之分布

Fig. 2. Profile distribution of labelled N in Hsueh-chia soil at 2 days after flood fertigation (^{15}N atom excess %×10^{-3}, mean of two replications)

結　論

尿素之淹施乃是不整地玉米之較佳施肥方式，惟淹施之適當水量有顯著的土壤間差異。滲透率低之土壤若淹施過量水分將增長脫氮機會。反之，滲透率高之土壤若淹水量過多則有污染地下水之嫌。霧峰和學甲之淹施水量各以 5mm 和 50mm 爲宜。

誌　謝

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Tracing of the Labelled Urea-N Flood-fertigated with Different Depths of Water at Knee-high Stage under No-till Conditions[1]

Shen Lian[2], Chong-Ho Wang[2] and Shui-Cheng Lee[3]

Summary

Five atom % ^{15}N-urea was used to study both the uptake by crop and the residual N in soil of the urea-N flood-fertigated with different depths of water (i.e,5mm,25mm50mm and 100mm) at the knee-high stage in no-till maize field. The rate of N applied was 70 kg/ha. A double flame was used to flood-fertigate the corn plant. In the subplot surrounded by the inner flame, the ^{15}N-urea together with the afore-mentioned depths of water was flood-fertigated, while in the plot between the outer and inner flames, the ordinary urea together with the same depth of water as in the inner flame was flood-fertigated. The plant uptake of labelled nitrogen as well as the distribution of residual ^{15}N in the soil profile were studied.

The percentage uptake of the urea-N differed significatly between the depths of water flooded and also between the locations.

At the loctions of Wu-feng, where the permeability of soil is classified as low to moderate, the uptake was the highest with the least amout of water (i. e., 5mm) added. The uptake reduced significantly in accordance with the increase in the depth of water flooded and was the least with 100mm flooding. The reduction perhaps was related to the more denitrification prevalent in the soil with greater depths of flood-fertigation.

At the locations of Hsueh-chia, where the permeability of soil is classified as moderate to well, the uptake was the lowest with the least amount of water flooded. The loss of nitrogen through volatilizion was expected to be high in these soils of higher pH, particularly with the surface application of urea. At these locations, the uptake was the highest with the 50-100mm flooding, as the urea applied was leached into the sub-surface layers of soils and the loss of nitorgen through volatization could be avoided. Denitrification is not prevalent in these soils due to the better aeration in these soils. The flood-fertigation with 100mm, however, was found apt to contaminate grownd water with nitrate, as the distribution of residual ^{15}N in the soil profile reveal such indication.

Key words : ^{15}N, Urea, Fertigation, Maize, N uptake.

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1. Contribution No. 1830 from Taiwan Agricultural Research Institute.
2. Respectively Soil Scientist and Assistant Soil Scientist, Taiwan Agricultural Research Institute. Wufeng Taichung, Taiwan. ROC.
3. Associate Chemist, Institute of Nuclear Energy Research, Atomic Energy Council, Executive Yuan. Lungtan, Taoyuan, Taiwan, ROC.

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隧道式設施及施用苦土石灰和稻殼對山葵產量與品質之影響[1]

王鐘和[2]　王貴美[3]　胡敏夫[4]

摘要：針對本省阿里山達邦地區山葵產量及品質均低之缺憾，本試驗探討隧道式設施及施用苦土石灰和稻殼對山葵產量及品質之影響：施用苦土石灰提昇達邦地區酸性土壤之 pH 值，顯著提高山葵全株鮮重及根莖鮮重之產量。施用稻殼之效果不顯著，可能係試區土壤有機質含量高及土壤經過翻鬆，各處理區總體密度均低之故。隧道式設施造成遮光率較高，致使冬季至春季山葵快速生長期間，山葵之生長量顯著低於無隧道式設施者。隧道式設施確有減輕傳播性病害之功能，顯著降低植體經由眞菌*Phoma wasabiae* Yokogi 感染而形成條斑病（streak disease）之程度。故達邦地區山葵生育過程中，因季節不同其適宜之遮蔽程度亦應不同，宜採機動之遮蔽方式，於夏季高溫時期設置簡易之隧道式設施或遮陰網以降低透光率，既有利於山葵生長，且可防止夏季多量雨水造成對植株之直接傷害及病害傳播。山葵根莖黑心症嚴重度（black rot severity %）及條斑病嚴重度（streak disease severity %）均與植體中鈣及硼之含量呈負相關。根莖黑心症嚴重度與條斑病嚴重度之間則呈正相關。

關鍵詞：苦土石灰、稻殼、隧道式設施、山葵。

山葵（*Wasabia japonica* Matsum）爲十字花科之多年生草本植物，喜陰溼環境，生育適溫爲8～18℃，通常被栽培於高冷地之衫木下。全株均具有獨特風味，以香、辛、甘及粘等四種特性傲居所有香辛料作物之上，非爲人工替代物所能比擬。含芥子油類與糖類結合成配醣體，各種酵素及維生素等，有促進食慾、防腐及抗菌特性，並有解毒、發汗、利尿及淸血等功用，常作爲吃生魚片之佐料[15]。本省山葵栽培歷史，經查考始於民國3年，由日人佐藤昌氏自日本引進至阿里山栽培，至今已80年[4,14]。依足立氏報告[15]日本旱田栽培山葵一般施肥方法爲基肥施用雞糞100kg/10a、熔磷40kg/10a及苦土石灰150kg/10a，均於種植前撒施，隨即翻犁混合。山葵種植後則施用化成肥料（含 N 14%、P_2O_5 14%、K_2O 14%）作爲追肥，春季追肥與秋季追肥同量，各爲160kg/10a。胡等[6]「山葵臺農1號之育成」報告中之肥料試驗資料，則指出化學肥料施用量最高區之產量最高，其用量爲施用臺肥複合1號900kg/ha（含 N 180kg/ha、P_2O_5 45kg/ha、K_2O 90kg/ha）。而本省農民在高利潤誘惑下，爲提高山葵之產量，多年來均施用大量肥料。據連氏及李氏[13]調查報告，阿里山地區農民栽培一作山葵之期間，所施用雞糞和複合肥料之量各達16 ton/ha 及11 ton/ha之譜。

1. 臺灣省農業試驗所　研究報告第 1831 號。
2. 本所農業化學系助理研究員。
3. 本所植病系助理。
4. 本所農藝系助理研究員。

據廖氏報告[14]阿里山地區栽培之山葵感染黑心病相當嚴重且普遍，其感病率高達100%，感病程度有輕有重，外銷日本必須切除罹病的部位，因此，外銷部份常只及採收量的30%，影響農民收益甚巨。本省農民一般所稱的黑心病，係指山葵根莖內髓（pith）組織部份具有黑色徵狀，其程度有輕有重，輕者呈黑灰色，重者則呈黑色，更嚴重時甚至腐爛呈黑色空心狀。根莖內黑灰色或黑色徵狀發生之範圍有大有小，大者幾乎佔滿整個根莖縱切面，輕者則成小點狀。仔細的檢視根莖縱切面可以發現部份根莖除了上述徵狀外，尚有一種維管束褐化之徵狀，日本栽植之山葵亦有此種情形。據報告[15]此種維管束褐化的現象係由一種眞菌類的病原菌 *Phoma wasabiae* Yokogi感染所致，日本稱之爲墨入病。一般由根莖受感染部位之維管束先褐化，並向上或向下延伸。維管束褐化之長度，有長有短，此種褐化之維管束，從根莖之縱切面觀察似褐黑色的線條，因此亦稱爲山葵條斑病（wasabi streak disease）[16]。根莖有上述徵狀時，農民不但要多僱工切除罹病的部位，增加工資支出，根莖產量也因而降低，並且亦因根莖一部份被切除，商品等級下降，造成每公斤售價下降，確實對農民的收益造成極大的損害。

因此，爲改善此種根莖普遍具有黑心病症之情形，探究形成之原因及解決之道。本試驗將根莖之病症區分爲黑心症（根莖內髓細胞部位具黑色徵狀者），及條斑病（維管束褐化者）兩種，探討其發生情形與營養狀況間之關係。

材料與方法

一、試驗地點之土壤理化性質及試驗處理

試驗前採自試驗區土壤之分析值列於表 1。表中表土及底土之 pH 值分別爲5.1與4.9，係強酸性土壤。據報告適宜山葵生長之土壤 pH 值爲6.0～7.0[15]，故爲求得中和土壤酸性至 pH 6.0所需之石灰需要量，並顧及強酸性土壤可能鎂含量不高，乃稱取土壤加入不同量之苦土石灰（比例爲0、1、2、3及4g. Kg^{-1}）混合均勻於250ml 之三角瓶中，置於恆溫箱中維持在30°C之狀況，孵育30日後測定pH 值，結果如圖1。圖1顯示施用苦土石灰3及4g·Kg^{-1}可提昇 pH 值至5.9及6.2，爲避免土壤 pH 值改變過劇，乃選擇施用3g·Kg^{-1}。因預計翻犁之深度爲20公分，且表底層土壤粒徑小於2mm 之部份約佔全土壤之一半（表1），故苦土石灰用量定爲3公噸／公頃。表土及底土小於2mm 之土壤，質地分別爲黏質壤土及坋質黏土，屬細質地土壤。由於栽培山葵理想之土壤爲排水良好之壤土或砂壤土[15]，故設計施用稻殼以改良土壤之物理性，以適宜山葵生長。另外爲防止傳播性病害之感染，部分處理則給予隧道式設施（寬2公尺、高1.5公尺）。處理內容如後；

表 1. 供試土壤理化性質

Table 1. Some physical and chemical properties of the experimental field

Item / Soil depth (cm)	Soil particle fraction (%) >4.75 (diameter; mm)	4.75～2	<2	Texture (<2mm soil) sand %	silt %	clay %	pH (1:1)	Organic matter %	CEC me/100g	Bray No. 1 P ppm	Mehlichs No. 1 K ppm	Hot water soluble B ppm
0—15	30.5	18.1	51.4	44.6	25.3	30.1	5.08	9.36	9.20	18.0	67.2	0.19
					Clay Loam							
15—30	32.0	19.6	48.4	16.9	40.2	42.9	4.88	5.85	8.76	13.5	70.5	0.18
					Silty Clay							

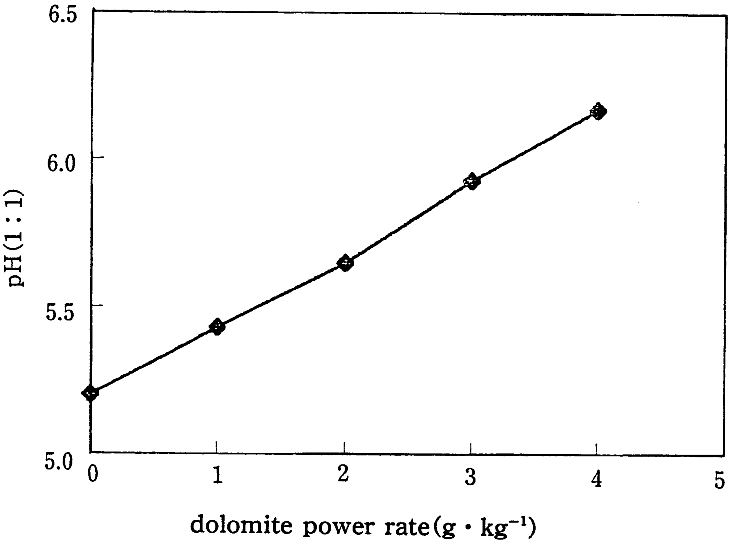

圖 1. 苦土石灰用量與 pH 值之相關
Fig. 1. Relationship between the rates of dolomite powder and the pH values of soil.

A. 有隧道式設施，施用苦土石灰（3t／ha）和稻殼（30t／ha）。
B. 有隧道式設施，僅施苦土石灰（3t／ha）。
C. 無隧道式設施，施用苦土石灰（3t／ha）和稻殼（30t／ha）。
D. 無隧道式設施，僅施苦土石灰（3t／ha）。
E. 慣行栽培法（無隧道式設施，不施苦土石灰和稻殼）。

各處理區均施用臺肥複合肥料5號（16：8：12）1,200kg/ha，施用方法乃50%當基肥，另外50%於隔年秋季時作爲追肥施用。各處理均依當地農民習慣施用有機質肥料，因爲表土及底土之有機質含量高達9.4及5.9%，故施用量較農民之慣用量減半，爲10ton/ha。A、B、C 及 D 各處理均施用之有機質肥料爲甘雨特肥，爲一種雞糞堆肥，內含 N 3.2%、P_2O_5 2.3%及 K_2O 2.5%，另外因製造過程中混合硼砂，故含有硼0.4%。E 處理區施用農民慣用之雞糞堆肥則未含硼砂，內含 N 3.6%、P_2O_5 2.9%及 K_2O 2.4%。苦土石灰、稻殼及有機質肥料均於種植前一個月撒施於土表，隨即翻犁（實際翻犁深度約25～30公分）。各小區均設置塑膠水管，於土壤乾燥時進行滴灌。

二、試驗設計：逢機完全區集排列，5處理，4重複。行株距40cm×20cm，每區種植4行，小區面積12m²（6m×2m），每小區種植120株，全部試區面積約240m²。

三、供試品種：山葵臺農1號。

四、栽培地點：阿里山鄉達邦村原住民保留地（海拔1,300m），試區土壤性質如表 1。

五、試驗期間：1990年10月至1992年6月。

六、調查項目：調查株高、葉數、分蘗數、全株及根莖產量，分析土壤理化性質[1]及熱水可溶硼含量[3,8]，測定試區透光度與植體成分分析。並調查葉、葉柄及根莖之品質及黑心症及條斑病發生情形，方法如下：

(一)a. 黑心症株百分率（black rot incidence %）；係收穫時每區調查10株，根莖縱切面具黑色徵狀之株數百分率。

b. 黑心症嚴重度（black rot severity %）：係將根莖縱切面黑心程度分爲5級；分別爲0級：無任何黑色徵狀者，1級：根莖縱切面黑色徵狀者面積佔全面積0～10%者，2級：根莖縱切面黑色徵狀面積佔全面積11～25%者，3級：根莖縱切面黑色徵狀面積佔全面積26～50%者，4級：根莖縱切面黑色徵狀面積佔全面積50%以上者，再依下式計算所得。

$$\text{黑心症嚴重度}=\frac{1\times n_1+2\times n_2+3\times n_3+4\times n_4}{4\times N}\times 100\%$$

N：調查根莖數

n_1, n_2, n_3, n_4：各級根莖數

(二)a. 條斑病株百分率（streak disease incidence %）；係調查根莖縱切面因眞菌 *Phoma wasabiae* Yokogi 感染而形成之維管束褐化，不論褐化之長短，只要具褐化現象，即予採計。調查方法爲收穫時每區調查10株，根莖縱切面具維管束褐化之株數百分率。

b. 條斑病嚴重度（streak disease severity %）；係將維管束褐化程度分爲5級；分別爲0級：無任何維管束褐化者，1級：根莖縱切面維管束褐化之長度佔全維管束長度0～10%者，2級：根莖縱切面維管束褐化之長度佔全維管束長度11～25%者，3級：根莖縱切面維管束褐化之長度佔全維管束長度26～50%者，4級：根莖縱切面維管束褐化之長度佔全維管束長度50%以上者。再依下式計算所得

$$\text{條斑病嚴重度}=\frac{1\times n_1+2\times n_2+3\times n_3+4\times n_4}{4\times N}\times 100\%$$

N：調查根莖數

n_1, n_2, n_3, n_4：各級根莖數

結果與討論

一、試區土壤 pH 值與水分含量之比較

表2顯示山葵種植時施用苦土石灰區之土壤 pH 值較對照區提高，分別爲表土由5.2提昇爲5.7（A、B、C 及 D 處理平均），提高0.5個單位。底土由5.3提昇爲5.7（A、B、C 及 D 處理平均），提高0.4個單位。沒有如實驗室內孵育試驗值高，可能係土壤翻犁之深度較深，達25～30公分之故。而且試區原有坡度25°，雖然經過延著等高翻犁整平，但試區內土壤之均匀度仍不及一般平地試區，因此 pH 值變異較大，未達顯著差異。收穫時表土之 pH 值則顯示有設施處理區之 pH 值略低於無設施處理，原因可能爲收穫期5月底之前較常下雨，土壤常保持濕潤，故 pH 值上昇。而 A 及 B 處理因有隧道式設施，阻隔部分雨水，土壤含水量較低，土壤較乾燥，致使表土之 pH 值較其它試區低。施苦土石灰之 C 及 D 處理區底土之 pH 值顯著地較同爲施苦土石灰 A 及 B 處理區高，原因可能爲經過長達約20個月之生育期，部份苦土石灰資材下移至底土層。而 A 及 B 處理區則因有隧道式設施，阻止苦土石灰被雨水淋洗下移，故底土之 pH 值低於 C 及 D 處理區。

表 3 土壤水分資料顯示，有隧道式設施之 A 及 B 處理區之土壤含水量除了在種植時及種植後414天採樣時適逢農民灌水，導致土壤含水量與無隧道式設施區相近外，其它時期土壤之水分含量大致均低於無隧道式設施處理區，此與前述隧道式設施阻隔部份雨水，使土壤含水量較低，土壤較乾燥之推論符合。表 4 爲試區之總體密度（bulk density; BD）資料，各處理區之總體密度均低，可能與試區土壤經過翻鬆及有機質含量高有關。惟施用稻殼之 A 及 C 處理區之表層及底層土壤之總體密度仍顯著地較無稻殼區低，顯示施用稻殼使土壤更加疏鬆。

表 2. 試區土壤 pH 值之比較

Table 2. pH value of experimental plot

Treatment	Surface soil pH (0～15cm)		Subsoil pH (15～30cm)	
	Planting	Harvest	Planting	Harvest
A	5.75a[z]	5.50b	5.35a	5.70b
B	5.45a	5.60b	5.88a	5.80b
C	5.79a	5.90ab	5.71a	6.20a
D	5.64a	6.00a	5.75a	6.30a
E	5.23a	5.90ab	5.26a	5.70b

[z] means with the same letter in each column are not significantly different at 5% by Duncan's multiple range test.

表 3. 試區土壤水分含量之比較

Table 3. Soil water content of experimental plot

Soil depth (cm)	Treatment	Soil water content (g•g^{-1} ; %)					
		planting[z]	86 DAP[z]	200 DAP	306 DAP	414 DAP	589 DAP
0～15	A	56.0a[y]	55.0a	54.5a	49.0b	63.8a	53.6a
	B	58.8a	53.3a	56.0a	51.0b	62.8a	52.2a
	C	56.8a	59.0a	62.3a	61.5a	64.8a	61.8a
	D	58.5a	56.8a	63.0a	59.3a	58.5a	62.6a
	E	63.3a	52.8a	67.5a	55.5ab	61.5a	59.2a
15～30	A	55.8a	56.0a	55.3a	48.0b	60.8a	54.7a
	B	60.3a	55.0a	61.0a	49.5b	64.8a	54.8a
	C	56.5a	59.3a	61.0a	66.5a	62.3a	55.8a
	D	59.0a	57.5a	66.0a	58.5a	63.5a	56.0a
	E	57.3a	53.0a	62.5a	58.8a	60.8a	54.7a

[z] Sampling time, DAP=Days after planting.
[y] means with the same letter in each column are not significantly different at 5% by Duncan's multiple range test.

表 4. 試區土壤總體密度之比較

Table 4. Bulk density of experimental plot

Treatment	Surface soil BD (g•cm^{-3}) (0～15cm)		Subsoil BD (g•cm^{-3}) (15～30cm)	
	Planting	Harvest	Planting	Harvest
A	0.56b[z]	0.82b	0.62bc	0.83c
B	0.68a	0.91a	0.67ab	0.87bc
C	0.57b	0.83b	0.61c	0.88b
D	0.66a	0.91a	0.71a	0.94a
E	0.62ab	0.93a	0.70a	0.97a

[z] means with the same letter in each column are not significantly different at 5% by Duncan's multiple range test.

二、產量與農藝性狀之比較

表 5 中各處理區植株之株高並無差異，施用苦土石灰處理區（A、B、C 及 D 處理）之分蘗株數目均較對照區（E）增加，C 及 D 處理分別增加達41%及38%，惟因變異大，未達顯著水準。單株可販售根莖數之比較，C 及 D 處理顯著地比對照區（E）多，平均多出53%，但同爲施用苦土石灰之 A 及 B 處理則無增加。其原因應爲 A 及 B 處理有隧道式設施，遮光比率各達86%及87%（表6），較僅依賴天然杉木林遮光 C 及 D 處理之71%顯著較高。以及A 及 B 處理區土壤較 C 及 D 處理區乾燥（表3），土壤 pH 值低於 C 及 D 處理（表2），較不利於山葵之生長。而 E 處理亦僅依賴天然杉木林遮光，因各試區內杉木生長之位置不一致，其遮光比率爲73%，略高於 C 及 D 處理，但統計上未達顯著差異。有關植株生長之情形將於下節中討論。同樣的，C 及 D 處理之全株鮮重收量也顯著地較對照區高（增加156%），及較 A 及 B 處理高（增加60%）。而 A 及 B 處理雖然較對照區高出約60%，惟未達顯著差異。根莖鮮重之收量資料則示施用苦土石灰之 A、B、C 及 D 處理區之收量均顯著地高於對照區，分別爲增加的18%（A 及 B 處理平均），及153%（C 及 D 處理平均）。又 C 及 D 處理與對照區（E）之差別除了苦土石灰及稻殼之有無，尙因 C 及 D 處理施用之雞糞堆肥內含有硼砂，因此該兩處理山葵生育及產量顯著較佳之部份原因，可能爲施硼之效果，有關各處理間植株硼含量之比較，將於後面文章中討論。無隧道式設施之 C 及 D 處理且顯著地高於有隧道式設施之 A 及 B 處理，達2倍以上。綜合以上產量資料，可看出施用苦土石灰提昇土壤 pH 值，顯著地促進屬十字花科植物山葵之生長，提高全株及根莖產量。有關施用石灰資材調整酸性土壤 pH 值至適宜作物生長之範圍，具有促進作物生長，增加作物產量之效果，常被提及[7,10,11,12]。而隧道式設施則不利於山葵生長，降低了苦土石灰之增產效果。表中各項資料亦顯示施用稻殼之 A 及 C 處理與無施稻殼之 B 及 D 處理間並無顯著之差異，其原因可能係試區土壤粒徑大於2mm 之石礫部份，接近50%，且有機質含量高（表 1），使土壤不致於太密實，以及各試區均經徹底翻鬆，土壤總體密度（BD; bulk density）均低（表3）之故。

表 5. 不同處理山葵之產量與農藝性狀

Table 5. Comparison of different treatments on the yield and agronomic of wasabi

Treatment	Plant height cm	Tiller no./plant	Salable rhizome no./plant	Whole plant fresh wt. ton/ha	Rhizome fresh wt. (ton/ha)
A	28.2a[z]	10.6a	1.4b	43.0b	2.86b
B	31.0a	9.0a	1.6b	40.8b	2.88b
C	33.2a	11.8a	2.3a	70.6a	6.48a
D	33.4a	10.9a	2.3a	61.8a	5.90a
E	31.8a	7.8a	1.5b	25.9b	2.45c

[z] means with the same letter in each column are not significantly different at 5% by Duncan's multiple range test.

表 6. 不同處理區之遮光比率

Table 6. Comparison of light shading percentage of experimental plot

Treatment	A	B	C	D	E
Light shading percentage %	86.4a[z]	86.5a	70.7b	70.7b	72.5b

[z] means with the same letter are not significantly different at 5% by Duncan's multiple range test.

三、生育期間各處理區植株鮮重之比較

圖 2 顯示有隧道式設施之 A 及 B 處理區之山葵，在冬季及春季植株快速生長期間，鮮重增加之速度明顯的較無隧道式設施之 C 及 D 處理低，顯然該時期高達86%之遮光率（表 6）不利於山葵生長。而 C 及 D 處理區因遮光率較低，平均達71%，透光率較高，山葵生育較佳。足立氏[15]指出日本山葵生長期間所需之遮光率不一，在六至八月高溫期間所需之遮光率最高，該時期遮光率達70%時，最適宜山葵生長。胡等[5]在露地以黑色PE遮陰網進行遮光試驗時，亦指出遮光率70%時，山葵之生育與收量優於遮光率爲48%、55%及62%之其它處理。本試驗則顯示在夏季高溫時期，C 及 D 處理因透光率較高，植株易產生嚴重軟腐乾枯，影響生育，導致鮮重下降之幅度大於 A 及 B 處理。此種情形與阿里山地區農民在高海拔（2,000m 左右）國有林班地種植之山葵之生長情形不同。連氏及李氏[13]指出高海拔國有林班地氣溫較低，山葵在冬季時生長停止，春季三月時則莖葉快速生長，同時伴隨著根莖肥大，至六月爲生長最茂盛期，七至九月時，因爲溫度昇高，氣候炎熱，不適於山葵生長，莖葉停滯不生長。而本試驗地點達邦地區原住民保留地之海拔僅1,300m，溫度較高海拔之國有林班地高，故夏季時造成植株生長停止，甚而使部份葉片乾枯及脫落，重量顯著減輕。而冬季時因溫度不致於過低，故植株仍能生長，春季時則如同林班地栽培之山葵一樣，莖葉快速而茂盛的生長(圖 2)。故在達邦地區合法之原住民保留地種植山葵時，生育過程中，因季節不同其適宜之遮蔽程度亦應不同，宜採機動之遮蔽方式，於夏季高溫時期設置簡易之隧道式設施或遮陰網降低透光率，既有利於山葵生長，且可防止夏季多量雨水造成對植株之直接傷害及病害傳播。

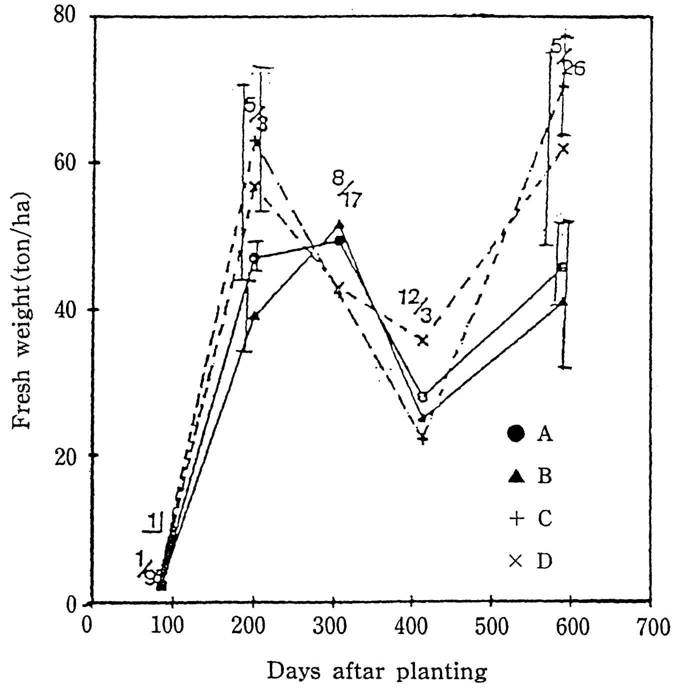

圖 2. 生育期間各處理之全株鮮收量之比較（註1：月／日）

Fig. 2. Comparison of different treatments on whole plant fresh weight at different growth period of wasabi (note 1: month/day)

四、山葵植株品質與鈣、鎂及硼含量比較

表7顯示各處理均有相當比例之根莖具黑色徵狀，經鑑定部份並兼具由眞菌*Phoma wasabiae* Yokogi 感染形成之條斑病（streak disease），其中尤其以 E 處理最嚴重，其黑心症株百分率（black rot incidence）達100%，黑心症嚴重度（black rot severity）亦達63%。其植體中之鈣、鎂及硼含量均顯著低於其它處理，顯示施用苦土石灰明顯地促進山葵對鈣素及鎂素之吸收。而有隧道式設施之 A 及 B 處理，植株地上部之葉（葉片＋葉柄）之鈣及鎂含量均顯著高於無隧道式設施之 C 及 D 處理，原因可能爲遮光率高，造成植株生長較慢，植株內含養分累積，因而濃度昇高。而 C 及 D 處理區則因植株生長快速，造成稀釋作用（dilution effect），使植體中養分濃度下降。又 A、B、C 及 D 處理均施用之有機質肥料爲甘雨特肥，內含有硼砂，而 E 處理施用農民慣用之雞糞堆肥則無。且試區土壤之熱水可溶硼素含量，表土及底土分別爲0.19 ppm 及0.18ppm（表 1），屬低硼含量之土壤[18]。故對照區植株硼含量較其它處理區低。

表 7. 根莖黑心症株百分率及黑心症嚴重度與植體鈣、鎂及硼含量之比較

Table 7. Comparison of calcium, magnesium and boron content on the rhizome black rot of wasabi

Treatment	Ca (%)		Mg (%)		B (ppm)		Black rot incidence (%)		Black rot severity(%)
	Leaf+ petiole	Rhizome+ root	Leaf+ petiole	Rhizome+ root	Leaf+ petiole	Rhizome+ root	tiller	rhizome	rhizome
A	2.80a[z]	0.92a	0.45a	0.28a	19.3a	13.8a	45.0ab	75.5b	36.5b
B	2.78a	0.81ab	0.45a	0.26a	19.7a	13.6a	27.5b	72.5b	36.2b
C	2.02b	0.80ab	0.34b	0.27a	21.2a	14.0a	43.4ab	80.0b	38.3b
D	1.76bc	0.66ab	0.32b	0.26ab	17.7ab	12.3ab	50.8ab	82.5b	37.5b
E	1.50c	0.57b	0.29b	0.22b	15.8b	10.8b	64.2a	100a	63.3a

[z] means with the same letter in each column are not significantly different at 5% by Duncan's multiple range test.

由於鈣及硼兩元素均與細胞之生長分化有密切之關係，鈣爲構成植物體中膠層之重要成分，在細胞膜或細胞壁存在較多，具有中和植物體內過剩之有機酸，強化細胞壁組織及調節體內水分等功能。移動性小，缺乏時新生部位易受影響，缺乏症狀有蕃茄尻腐症，白菜和芹菜之心腐症等。硼則與細胞分裂，花粉受粉，養分吸收及糖分之輸送等有密切關係，移動性亦小，故缺乏時生長點之生長停止，變脆、變黃而枯死；根部或莖部之中心部分變黑，根莖類缺硼時易生心腐病，缺硼之症狀與缺鈣者相似[2,3,17]。而本試驗中山葵葉片及葉柄內鈣含量爲1.5～2.8%，根莖及根則爲0.6～0.9%，均明顯低於日本所種植山葵之鈣含量（分別爲：葉片及葉柄約3.0～4.0%，根莖約1.0～1.6%）[15]。葉片及葉柄之硼含量爲16～21ppm，根莖及根之硼含量則僅爲11～14ppm，均甚低，雖缺乏前人有關山葵硼含量之資料可資比較，惟與同屬十字花科植物硼含量之22～65ppm 比較，則明顯偏低[2]。本試驗中山葵根莖及根之鎂含量爲0.22～0.28%，與日本山葵根莖鎂含量約0.25相近[15]。鎂爲葉綠素之成分，與葉片光合作用有密切關係，在植物體內移動性大，故缺鎂時徵狀先呈現於老葉，由葉脈間黃化，與葉脈周圍之綠色成明顯對比[2,9,17]。而本試驗中，山葵生長之期間，並未觀察到植株老葉有任何缺鎂之症狀，即使是對照區亦是如此。由於本試驗中山葵植體內鈣及硼含量均明顯偏低，考量鈣素及硼素之營養生理功能，及缺乏時產生之症狀，研判與根莖黑心症之產生有關聯。當然也會影響山葵之生育與產量。

表 8 中根莖條斑病之感染情形，以對照區最爲嚴重。施用苦土石灰且無隧道式設施之 C 及D 處理區根莖之條斑病嚴重度顯著地較對照區低，條斑病株百分率亦較對照區略低，其它項目則無差異。值得注意的是，有苦土石灰及隧道式設施之 A 及 B 處理，植株之葉部、分蘗苗及根莖之條斑病株百分率及條斑病嚴重度不但均顯著地較對照區低，也顯著較同樣施用苦土石灰但無隧道式設施之 C及 D 處理低，顯示隧道式設施確實具有防止病害傳播之功能。

表 8. 山葵植體由眞菌*Phoma wasabiae* Yokogi 感染形成條斑病之評估

Table 8. Evalution of streak disease caused by *Phoma wasabiae* Yokogi on the wasabi

Treatment	Disease incidence of leaf (%)	Disease severity of leaf (%)	Disease incidence of tiller (%)	Disease incidence of rhizome (%)	Disease severity of rhizome (%)
A	37.1b[z]	11.8b	20.0b	30.0c	6.7c
B	44.1b	15.9b	18.3b	32.5c	7.8c
C	71.8a	33.6a	32.5a	70.0ab	17.0b
D	66.0a	31.9a	33.5a	50.0bc	12.5bc
E	67.7a	31.6a	40.8a	92.5a	34.0a

[z] means with the same letter in each column are not significantly different at 5% by Duncan's multiple range test.

圖 3 係根莖及根之鈣含量與根莖黑心症嚴重度及條斑病嚴重度之相關圖，顯示鈣含量與兩者間均具負相關。圖 4 則顯示硼含量亦與兩者間均存在有負相關。硼含量與黑心症間呈極顯著之負相關，亦即根莖硼含量愈低，根莖黑心症嚴重程度愈高。圖 5 中根莖黑心症嚴重度與條斑病嚴重度之間呈顯著正相關。顯示根莖罹患黑心症之程度愈高，其受眞菌 *Phoma wasabiae* Yokogi 感染而形成條斑病之維管束褐化程度也愈嚴重。綜合前面各項結果。可知鈣及硼營養與山葵根莖產生黑心症及條斑病間有密切相關。彼此間之詳細關係如何，値得進一步加以探討釐清。

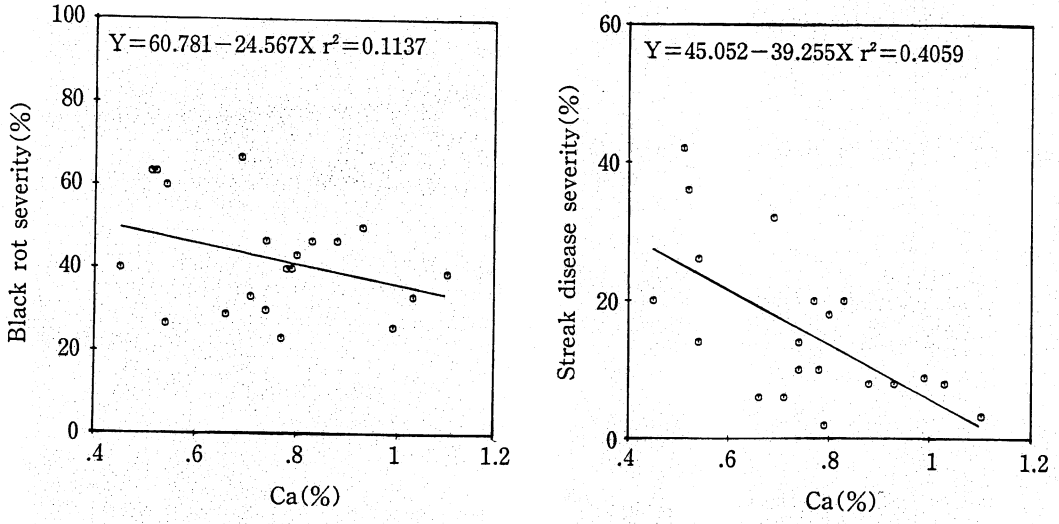

圖 3. 山葵根莖及根之鈣含量與根莖黑心症嚴重度及條斑病嚴重度之相關

Fig. 3. Relationship between calcium content (in rhizome+root) and rhizome black rot severity or streak disease severity of wasabi

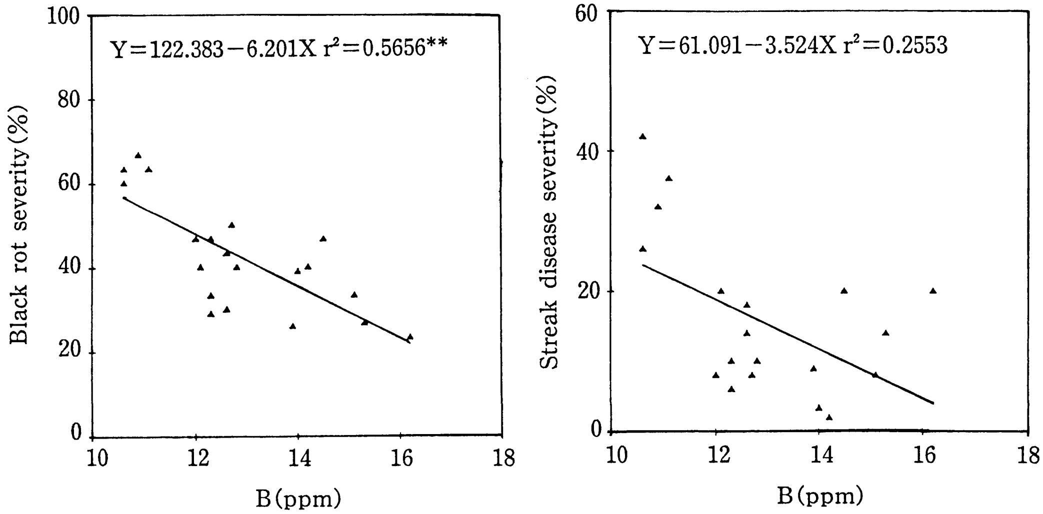

圖 4. 山葵根莖及根之硼含量與根莖黑心症嚴重度及條斑病嚴重度之相關

Fig. 4. Relationship between boron content (in rhizome+root) and rhizome black rot severity or streak disease severity of wasabi

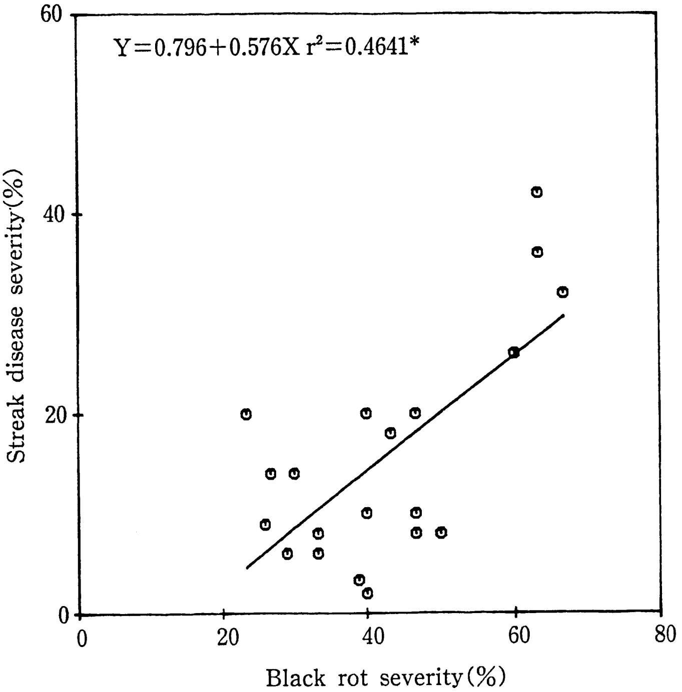

圖 5. 山葵根莖黑心症嚴重度與條斑病嚴重度之相關

Fig. 5. Relationship between the rhizome black rot severity and the streak disease severity of wasabi

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Effect of Tunnel System, Dolomite Powder and Rice Hull on the Yield and Quality of Wasabi (*Wasabia japonica* Matsum)[1]

Chong-Ho Wang[2], Kuei-Mei Wang[3] and Min-Fu Hu[4]

Summary

The purpose of this study was to investigate the effect of applied dolomite powder, rice hull and tunnel system on the yield and quality of wasabi (*Wasabia japonica* Matsum) . Some of the results were summarized as follows: Applied dolomite powder increased soil pH value, that also increased whole plant fresh weight and iccreased salable rhizome yield significantly, applied rice hull had no positive effect on plant growth. Tunnel system induced higher light shading, conducted to lower growth rate at winter and spring season, resulted in a lower yield. Tunnel system could avoiding the quality decreased dued to streak diseased caused by *Phoma wasabiae* Yokogi. Rhizome black rot severity and streak disease severity both had a negative relation with the calcium or boron content in wasabi. Rhizome black rot severity also had a positive relation with the rhizome streak disease severity of wasabi.

Key words : Dolomite powder, Rice hull, Tunnel system, *Wasabia japonica* Matsum.

1. Contribution No. 1831 from Taiwan Agricultural Research Institute.
2. Assistant Soil Scientist, Department of Agriculture Chemistry, TARI. Wufeng, Taichung, Taiwan, ROC.
3. Assistant, Department of Plant Pathology, TARI.
4. Assistant Agronomist, Department of Agronomy, TARI.

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非洲菊斑潛蠅之形態、生活史及繁殖力[1]

錢景泰　古琇芷[2]

摘要：本試驗以菜豆苗飼育非洲菊斑潛蠅，並觀察該蠅之形態、生活史及交尾次數與溫度對其族群增長之影響。結果得知，成蠅之羽化、產卵、取食及老熟幼蟲鑽出葉片等活動均屬晝行性。羽化後第二日之交尾率即達 99%，雌雄蠅皆行多次交尾（4～20 次以上），在 25°C下、未交尾雌蠅雖可產少數之卵，但均不孵化，而僅交尾 1 或 1 次以上雌蠅之內在增殖率各爲 0.2301與 0.2329／日。雌蠅可自取食孔或產卵孔取食，其中在產卵孔處之吸食可提高卵之孵化。雌蠅對豆苗之眞葉與複葉間具極顯著之產卵與取食偏好性，完全不產卵於複葉。卵之形成屬應變式產卵策略，卵在發育期不增大。於 20°～35°C 時，可利用豆葉內第三齡幼蟲數估算該蠅之產卵數(孵化率 94～99%，幼蟲存活率 90～98%)。第三齡幼蟲之食痕長度各爲第一與第二齡幼蟲之 9.0 與 4.7 倍。雌蠅產卵之實測臨界低溫與臨界高溫各爲 12°C 與 40°C。卵至成蟲期之發育臨界低溫與實測致死高溫各爲 8.7°C 與 38°C。餵食蜂蜜時，35°C 爲該蠅族群增長最大之溫度(內在增殖率爲 0.3236／天)；15°C 時族群幾停止增長(內在增殖率爲 0.0051／天)；而 20°C 至 25°C 爲該蠅最適繁殖之溫度(淨增殖率爲 152～188 粒雌性有活力之卵／雌)。在 25°C 下，一代發育期 16.6 天(卵期 3.3 天、幼蟲期 4.6 天、蛹期 8.6 天)，一代存活率 81.6%。在 25°C 室溫下、每日供食蜂蜜、並以 15～20 cm 高之菜豆苗供該蠅產卵爲最佳飼養條件。

關鍵詞：非洲菊斑潛蠅、生活史、族群增長、菜豆、溫度。

非洲斑潛蠅（*Liriomyza trifolii* (Burgess)）爲全球性觀賞植物與蔬菜之重要害蟲[15]。由於其繁殖力高、發育期短及易產生抗藥性等特性致使藥劑防治不易[21]，但一些學者認爲在觀賞植物上可利用寄生蜂防治該蠅[13,16,20]。由於非洲菊斑潛蠅之繁殖力受到寄主植物種類、葉品質、溫度、食物及該蠅之品系等之影響[16]，因而在評估寄生蜂對非洲菊斑潛蠅之防治效果前，必先瞭解非洲菊斑潛蠅在不同寄主植物上之生物學。然至今有關該蠅生物學研究多在菊花、芹菜、番茄及一種菜豆（*Phaseolus* sp.）上進行[8,10,14,18,20,23,26]，且對雌蠅之交尾能力、交尾次數與溫度對該蠅族群增長率之影響等均未有深入研究。1988 年 2 月臺灣首次於臺中大坑地區之非洲菊園（*Gerbera jamesonii* Bolues ex Hook. f.）發現非洲菊斑潛蠅，隨後該蠅迅速擴散分布並危害各地之菊科花卉、豆科、葫蘆科、茄科及十字花科蔬菜[3,27]。經調查在臺灣非洲菊斑潛蠅本地種之寄生蜂共有 7 種，其中以異角釉小蜂（*Hemiptarsenus varicornis* (Girault)）最爲常見[9]。本文爲深入瞭解非洲菊斑潛蠅之生活習性與繁殖潛能，乃以菜豆（field bean, *Phaseolus vulgaris* var. *communis* Aeschers）爲寄主植物，觀察該蠅之形態、生活史及交尾次數與溫度對族群增長之影響等，希冀提供該蠅繁殖方法及評估黃色黏板與寄生蜂二者對非洲菊斑潛蠅防治潛能之參考。

1 臺灣省農業試驗所　研究報告 1832 號。
2 本所應用動物系副研究員、計畫助理。臺灣省　臺中縣　霧峰鄉。

材料與方法

一、寄主植物之栽培

每週定期浸泡菜豆種子(600 粒)，在 25°C下經 7 小時種子吸水飽滿後，將其瀝淨、並風乾 2 小時，其後再以濕布覆蓋催芽，待 14 小時種子發根後，即移種在溫室置有 3 號蛭石之穴盤(長 36.5 cm，寬 28 cm，高 4.5 cm；30 穴)內。每天澆水，經 3 ～ 4 天莖高 2 cm 時才在每一穴盤下另墊盛水之塑膠盤(長 49 cm，寬 34 cm，高 3.5 cm)，隔天灌水之。又經 10 ～ 13 天，菜豆苗長至高 15 ～ 20 cm、眞葉(primary leaf)葉寬達 7 ～ 9 cm 時，即可供室內非洲菊斑潛蠅產卵用。

二、供試蟲源

在臺中大坑非洲菊栽培園內採集被非洲菊斑潛蠅幼蟲危害之葉片，攜回室內先分裝於扎孔之塑膠袋(長 36 cm，寬 24 cm)，再置於 25°C 之定溫箱內，成蠅羽化後供做飼育之蟲源。

三、非洲菊斑潛蠅之形態與發育經過

在 25°C下將第二日齡之雌蠅接入內置有 20 株菜豆苗之繁殖網箱(長 75 cm，寬 55 cm，高 50 cm；網目 92 meshes)，經產卵 2 小時(上午 8 點至 10 點)後將菜豆苗移出。繼之在溫度 25°C、相對濕度 65 ～ 85%RH 及光周期 14 ： 10 (L：D)(上午 5 點至下午 7 點間照光)下，每日觀察該蠅各生長期之形態與發育經過，各做 20 ～ 60 隻。各齡期幼蟲頭咽骨長度之測量，需先製成玻片，再藉立體顯微鏡中之測微尺測量。而各齡期幼蟲食痕寬度之測量亦在立體顯微鏡下進行，至於各齡期幼蟲食痕長度之度量，則係先在一般縫衣用之細線上塗以一薄層經 5 倍水稀釋後之南寶樹脂，然後將該細線沿著各齡幼蟲之食痕輕輕地逐一黏著，待完成後剪斷此線段，並將其拉直測量其長度，各做 30 次。

四、非洲菊斑潛蠅成蠅之習性

羽化：1992 年 8 月 22 至 26 日與 1994 年 5 月 17 至 20 日分別在 25°C、相對濕度 65 ～ 85% RH 及自然光照下，觀察室內所飼養之非洲菊斑潛蠅成蠅之羽化方式並記錄一日內該蠅每小時之羽化頻率及性比。兩次觀察蟲數各爲 1,364 與 2,504 隻。

交尾：在前述相同溫、濕度與 1994 年 5 月 18 至 20 日自然光照條件下，將早上 6 點至下午 5 點間每小時所羽化之成蠅各自接入玻璃筒(直徑 20 cm，高 22 cm)內且供食純蜂蜜做群體飼育。當目視玻璃筒內雌雄蠅有交尾行爲時，利用小毛筆將成對成蠅移入指形管(直徑 1.5 cm，高 7 cm)內，觀察成蠅交尾之方式與時間，各觀察 30 對；或利用吸蟲管直接將正交尾之成蠅吸出，記錄其交尾前期與第二日齡成蠅於一日內每小時之交尾頻率等。各觀察 391 與 356 對成蠅。另在相同溫度與自然光照下，將一日內羽化成蠅接入上述玻璃筒，供食純蜂蜜做群體飼育，記錄各日齡成蠅之存活率及檢視雌蠅各日齡之交尾率與交尾次數。成蠅日存活率之試驗，每玻璃筒中各接入 50 對成蠅，共重複 6 次。而雌蠅各日齡之交尾率與交尾次數之試驗，係先準備各貼有 0 、1 、2 、3 及 4 次不同交尾次數標籤之 5 個大小相同之上述玻璃筒，然後將初羽化第一日齡成蠅接入標有 0 次交尾標籤之玻璃筒內。當目視玻璃筒內之成蠅有首次交尾行爲時，即利用吸蟲管將成對之成蠅吸入大玻璃管(直徑 3 cm，高 20 cm)內，且不驚動其交尾。待其自然分開後，再接入貼有 1 次交尾標籤之玻璃筒內。若在貼有 1 次交尾標籤之玻璃筒內目視成蠅有再次交尾行爲時，亦利用上述相同方法，將成蠅接入貼有 2 次交尾標籤之玻璃筒內。以此類推，即可得知第一日齡雌蠅之當日交尾率與交尾次數。另每日下午 5 點將供試之雌、雄蠅分置於不同之玻璃筒內，至次日早上 8 點再將供試雌雄蠅共置貼有 0 次交尾標籤之玻璃筒內，繼續觀察雌蠅各日齡之交尾率與交尾次數。各觀察 60 ～ 395 隻成蠅與 34 ～ 356 次交尾次數。

產卵：在 25°C、相對濕度 65 ～ 85% RH 及光周期 14 ： 10 (L：D)(上午 5 點至下午 7 點間照光)下，將目視已交尾第二日齡雌蠅 1 隻接入玻璃筒(直徑 12 cm，高 21 cm)，並供應 1 株帶有複葉與眞葉之菜豆苗，觀察雌蠅產卵之方式與時間、及計數雌蠅 1 天內在複葉與眞葉上之實際產卵數。共觀

察 20 重複。雌蠅產卵時刻之觀察則在上述相同條件下，自上午 5 點起進行。其間除下午 5 點至 7 點與下午 7 點至次日上午 5 點間係分別隔 2 與 10 小時供應 1 株去除複葉之菜豆苗外，其餘時刻均係每隔 4 小時更換菜豆苗，供 1 對第二日齡之非洲菊斑潛蠅產卵，共持續 48 小時。然後利用鏡檢法計數雌蠅在第二與第三日齡時在各處理時段內之實際產卵數，共做 13 重複。

取食：觀察雌蠅取食之方式、時間、部位及活動時刻等，其方法與前項產卵試驗相同。至於雌蠅在產卵孔處之取食對卵孵化之影響，此試驗係在 25°C下，當雌蠅產卵後，欲在其產卵處取食時，以細毛筆將其移位，並以油性簽字標記該處，4 天後觀察卵之孵化情形。每次觀察 16 ～ 28 粒卵，共做 8 重複。而雄蠅取食方式之觀察，係先將已被 20 隻雌蠅取食與產卵 1 分鐘後之菜豆苗移入玻璃筒(直徑 12 cm，高 21 cm)內，再接入 1 隻初羽化尙未進食之雄蠅，觀察其 10 分鐘內之取食行爲。共做 34 次。

雌蠅交尾次數與族群增長：試驗分三種處理進行，即依前項中有關對該蠅交尾前期與終生交尾次數等之瞭解，當目視雌蠅一羽化，即將其自群體中分離者，爲未交尾者；當目視雌蠅與雄蠅一發生首次交尾行爲後，即將雌蠅單獨分離，或將雌、雄蠅成對終生永置一起者，則各視爲祗交尾 1 次或 1 次以上之雌蠅。試驗進行時係在前述相同溫濕度、光照及空間(直徑 12 cm，高 21 cm 之玻璃筒)條件下，自雌蠅(體長＞ 1.9 mm)羽化當天起，每處理每日除於玻璃筒內壁以細毛筆塗畫方式供食純蜂蜜外，尙供應 1 株去除複葉之菜豆苗，直至雌蠅死亡爲止。記錄其間各處理雌雄蠅之壽命、實際產卵數或產有活力之卵數(viable eggs)、取食刻點數(雌蠅在豆葉上危害形成之所有白色刻點數)、及子代之成蠅數與雌性比(♀／♀＋♂)。其中雌蠅每日產有活力之卵數，係以卵產後 5 天，卵已發育爲第三齡幼蟲之數目計算之。每一處理供試之雌蠅或雌雄蠅均爲 1 隻或 1 對，共進行 6 ～ 10 重複。同時將上述資料及該蠅卵在 25°C 下之發育期與存活率資料，代入 Birch 之公式[7]，以估算雌蠅在不同交尾頻率下之族群介量，如內在增殖率、終極增殖率、淨增殖率及平均世代時間等。

五、溫度對非洲菊斑潛蠅發育之影響

利用非洲菊斑潛蠅於 25°C 下各在菜豆苗上所產下 2 小時與 24 小時內之蠅卵，進行九種不同溫度對該蠅發育之影響。亦即將各帶有 8 ～ 13 粒蠅卵之菜豆苗各放入 10°、12°、15°、20°、25°、30°、35°、38° 及 40°C 之定溫箱內。就產卵經 2 小時之卵，每日觀察溫度對該蠅各蟲期與卵至成蟲期之發育日數與存活率之影響，並在近各蟲期或齡期變化之際，每小時記錄該蠅之發育情形。而經產卵 24 小時內之卵，則在不同溫度下，僅觀察一個世代之發育日數與存活率。其中就發育期各觀察 6 ～ 56 隻，就存活率各做 5 ～ 10 次不等。並依 Campbell *et al.* 之方法[9]，估算該蠅之發育臨界低溫與發育有效積溫。

六、溫度對非洲菊斑潛蠅族群增長之影響

利用在 25°C 下非洲菊斑潛蠅初羽化且於下午 3 點前目視已完成交尾體長大於 1.7 mm 之成蠅，各取 1 對接入玻璃壁塗有純蜂蜜之玻璃筒(直徑 12 cm，高 21 cm)內，並供應 1 株去除複葉之菜豆苗，然後放置於前述 12°～ 40°C 間之八種不同定溫下。每處理組每日早上 7 點除將已被產卵或取食過之菜豆苗移出並更換新鮮之 1 株豆苗外，尙供食純蜂蜜，直至雌蠅死亡爲止。並記錄各處理 1 對成蠅之壽命、產卵前期、產卵期、實際產卵數或產有活力之卵數及取食刻點數。各進行 5 ～ 19 重複。同時將各處理每日移出帶有蠅卵之菜豆苗均移至 25°C 下飼育，直至羽化，並區分雌雄性。然後利用前項非洲菊斑潛蠅於 24 小時內之蠅卵在各不同溫度處理中之發育期與存活率資料及本項試驗所得之資料，代入 Birch 之公式[7]，以估算該蠅在各不同定溫下之族群介量，如內在增殖率、終極增殖率、淨增殖率及平均世代時間等。

七、低溫對非洲菊斑潛蠅蛹貯存之影響

將 25°C 下發育之非洲菊斑潛蠅第一日齡蛹 20 個(體長＞ 1.6 mm)分別裝入指形管(直徑 1.5 cm，高 7 cm) 內，並直接放置於 8°、12° 及 15°C 等三種不同之定溫箱內，待貯存 0 至 4 週後，再移至

25°C 定溫下記錄各處理成蠅之羽化率，各做 3 重複。

試驗結果經變方分析(ANOVA)，再以最小顯著差(LSD)法或 t 值測驗法比較處理間之差異性，顯著水準爲 5 %，遇百分率時，資料先予轉換(arcsin $\sqrt{x}$)再進行分析。

結 果

一、非洲菊斑潛蠅之形態與發育

(一)形 態

卵(圖 1 -B)：初乳白色半透明，經 48 小時後變無色透明。橢圓形。長 0.243 ± 0.002 mm，寬 0.134± 0.004 mm(n= 60)。

幼蟲共 3 齡。初無色，後隨成熟而呈黃色。蛆狀，外表光滑。頭部較尖，腹部末端較鈍。頭咽骨明顯，可藉由其大小識別各齡期。第一至第三齡幼蟲之特徵分述如下。

第一齡幼蟲：無色透明。體長(初～末期) 0.34 ± 0.01 ～ 0.73 ± 0.01 mm，寬(初～末期) 0.12 ± 0.01 ～ 0.25 ± 0.01 mm(n= 20)。頭咽骨長 0.103 ± 0.002 mm(n= 30)。

第二齡幼蟲：淡黃色。體長(初～末期) 0.84 ± 0.01 ～ 1.33 ± 0.01 mm，寬(初～末期) 0.29 ± 0.01 ～ 0.44± 0.01 mm(n= 20)。頭咽骨長 0.169 ± 0.002 mm(n= 30)。

第三齡幼蟲(圖 1 -D)：淡黃至黃色。體長(初～末期) 1.57 ± 0.01 ～ 2.55 ± 0.05 mm，寬(初～末期) 0.51 ± 0.01 ～ 0.76 ± 0.01 mm(n= 20)。頭咽骨長 0.250 ± 0.002 mm(n= 30)。

蛹(圖 1 -E)：褐色。長橢圓形，外有環節。體長 1.86 ± 0.02 mm，寬 0.89 ± 0.01 mm(n= 40)。

成蠅(圖 1 -A)：體黑色。複眼紅色；觸角，中胸小楯片，足基節、腿節，及平均棍均爲黃色；脛節、跗節棕色；腹部背板具明顯黃色中溝與邊緣區。翅前緣脈延伸至中脈 M_{1+2}，前緣脈區段比值爲 2.6 ： 1 ： 0.88 ，內橫脈約位於翅中室中央。雌體長 1.89 ± 0.01 mm，寬 0.57 ± 0.03 mm(n= 30)。雄體長 1.62 ± 0.02 mm，寬 0.55 ± 0.01 mm(n= 30)。

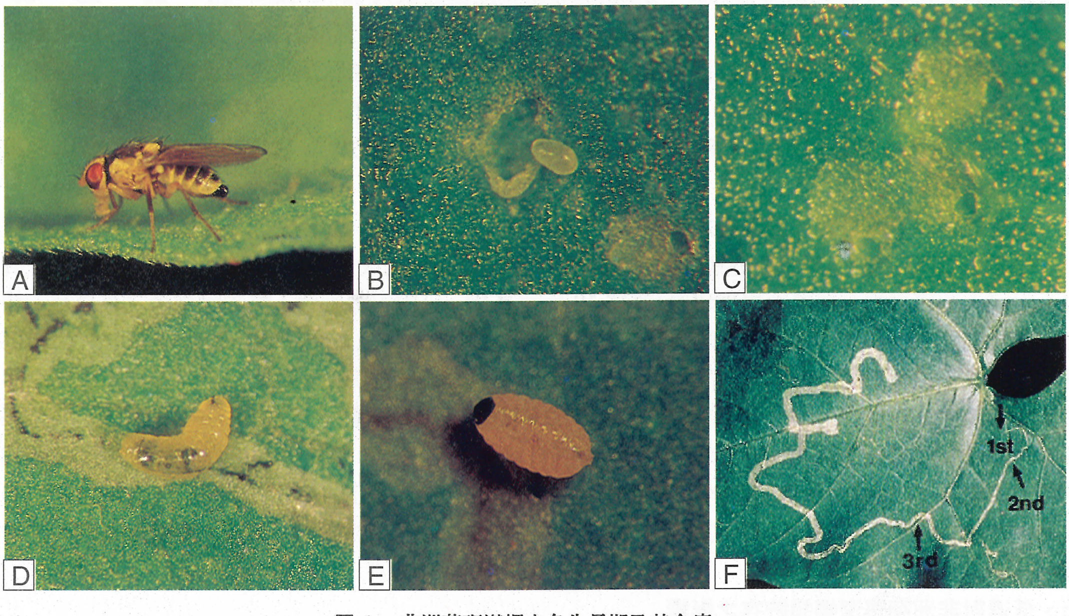

圖 1. 非洲菊斑潛蠅之各生長期及其食痕。

Fig. 1. Growth stage of *Liriomyza trifolii* and its serpentine mine.
A: Adult; B: Egg and oviposition site; C: Feeding puncture; D: The third instar larva; E: Pupa; F: Serpentine mine of larva.

(二)發育經過

在25°C下，非洲菊斑潛蠅自卵發育至成蠅羽化需16.6天。其中卵期爲3.3天，幼蟲期爲4.6天，第一至第三齡幼蟲之發育期各爲0.9、0.9及2.8天，蛹期爲8.6天(表1)。該蠅一代存活率爲81.6%，其中卵之孵化率爲99.0%，幼蟲與蛹之存活率各爲97.8與84.5%(表2)。卵發育中卵形未增大，近孵化時可自近產卵孔開口處卵之一端內看到第一齡幼蟲之褐色頭部與黑色角質化之頭咽骨片，孵化時，第一齡幼蟲在卵內先行180°之轉動，然後咬破卵殼，背向產卵孔開口之方向，在寄主葉表皮下利用大顎在柵狀葉肉(palisade mesophyll)層中取食並蠕動蛇行。至第二或第三齡幼蟲時，有時受食物量之限制，幼蟲亦可在海綿葉肉(spongy mesophyll)層中取食。其食痕初呈無色，隨後轉白色，同時在食痕隧道兩側交互留有明顯之黑色排泄物。幼蟲之食痕形狀與寄主植物之種類與品種有關。在菜豆上，食痕一般呈蜿蜒狀(serpentine shape)，有時亦呈大斑點狀(blotchlike)。第一齡幼蟲食量小、食痕細窄，長1.1 ± 0.1 cm、寬(初～末期) 0.136 ± 0.003 ～ 0.36 ± 0.01 mm。至第二與第三齡時，食量增大，食痕乃漸寬。其中第二齡之食痕長2.1 ± 0.1cm、寬(初～末期)0.44 ± 0.01～

表 1. 不同定溫下非洲菊斑潛蠅卵、幼蟲及蛹期之發育日數

Table 1. Duration in days for preimagial stage of *Liriomyza trifolii* at various constant temperatures

Temp. (°C)	Egg[z]		Larva[z] 1st		Larva[z] 2nd		Larva[z] 3rd		Pupa[z]		Egg-adult[z]	
	N[y]	x̄±SEM	N[y]	x̄±SEM	N[y]	x̄±SEM	N[y]	x̄±SEM	N[y]	x̄±SEM	N[y]	x̄±SEM
10	34	14.1±0.10a	25	7.60±0.30a	21	8.10±0.20a	17	13.30±0.30a		—		—
12	35	12.5±0.20b	29	5.30±0.20b	25	6.80±0.20b	25	11.00±0.30b	6	49.7±1.4a	6	89.7±1.4a
15	56	9.1±0.10c	42	4.93±0.12c	42	4.48±0.10c	42	8.64±0.10c	11	41.9±0.5b	11	68.1±0.5b
20	49	4.2±0.10d	41	2.01±0.02d	38	2.21±0.02d	37	2.90±0.03d	35	10.4±0.1c	35	21.4±0.2c
25	54	3.3±0.00e	50	0.84±0.01e	50	0.86±0.01e	48	2.80±0.04d	48	8.6±0.2d	38	16.6±0.1d
30	49	2.1±0.04f	47	0.80±0.01e	47	0.88±0.01e	47	2.00±0.01e	47	7.2±0.1e	38	13.2±0.1e
35	41	1.8±0.10g	32	0.83±0.02e	29	0.85±0.03e	29	1.30±0.03f	30	6.5±0.1f	28	11.7±0.1f
38	37	1.7±0.02g		—		—		—		—		—

[z] Means in each column followed by the same letter are not significantly different at 5% level by LSD.
[y] N: number of observation.

表 2. 不同定溫下非洲菊斑潛蠅卵、幼蟲及蛹期之存活率

Table 2. Survival rate of preimagial stage of *Liriomyza trifolii* at various constant temperatures

Temp. (°C)	% Survival(x̄±SEM)[z] Egg	Larva	Pupa	Egg-adult
10	65.3±2.4c	22.9±4.0e	0c	0c
12	77.4±2.3bc	49.1±4.1d	21.8±3.9b	8.0±1.4b
15	84.4±3.7b	89.0±3.1c	19.5±7.0b	13.9±4.9b
20	95.0±3.3a	90.1±4.6bc	88.9±3.3a	76.2±6.2a
25	99.0±1.0a	97.8±1.5a	84.5±4.7a	81.6±4.5a
30	93.8±4.3a	96.6±2.2ab	83.1±5.6a	74.5±4.3a
35	97.6±1.2a	96.6±0.6abc	80.8±3.1a	75.4±2.6a
38	68.2±1.6c	0f	—	—
40	0d	—	—	—

[z] Means in each column followed by the same letter are not significantly different at 5% level by LSD. Data were transformed to arcsin $\sqrt{x}$ prior to ANOVA. Field bean with 8—13 *L. trifolii* eggs per leaf, 5—10 replications.

0.80 ± 0.01 mm，第三齡之食痕長 9.9 ± 0.4 cm、寬(初～末期) 0.83 ± 0.01 ～ 1.79 ± 0.04 mm(圖 1 -F)。第三齡幼蟲老熟時視其在葉肉內潛行之位置，將食痕末端或近末端之葉上表皮或下表皮咬破一半圓形，然後自葉內鑽出，此時老熟幼蟲依其之趨暗性與趨觸性，大多滾落土中或暗處化蛹，少數者因葉面較平未滾落土中乃於葉面化蛹。 88%之第三齡老熟幼蟲在上午 8 點至 11 點間鑽出葉面而化蛹，而上午 11 點至翌晨 8 點間鑽出者僅 12%(n= 855)。化蛹初期蛹淡褐色，後轉褐色，至羽化前，蛹體呈黑褐色。

㈢成蠅之習性

羽化：成蠅羽化時在蛹殼內先利用頭部之撞擊，並藉其前額囊(ptilinum)之膨脹與收縮將蛹殼前端頂破爬出，其過程約需 5 ～ 20 分鐘。初羽化成蠅具向光性，先爬向玻璃筒壁上方或植株上，然後靜止不動，經 25.0 ± 2.5 分鐘(n= 47)後，前翅展開。再經 69.0 ± 0.7 分鐘(n= 48)，體軀骨化完全、體色加深。成蠅羽化盛期之時刻受黎明時間早晚之影響。在 5 與 8 月時成蠅之羽化時刻雖均在上午 4 點至下午 5 點之間，但其羽化盛期之時刻， 5 月時集中在上午 7 點至 11 點間(62%)，而在 8 月時則提前爲上午 5 點至 8 點間(51%) (圖 2)。雄蠅之羽化時刻較雌蠅稍早，如 5 月時上午 7 時至 9 點間當 39%雄蠅已羽化時，雌蠅之羽化率僅達 22%；而 8 月時，上午 5 點至 7 點間雄蠅羽化率達 40 %時，雌蠅之羽化率僅 35%(圖 2)。5 與 8 月時雌雄蠅之性比各爲 0.51 ： 0.49 與 0.53 ： 0.47。

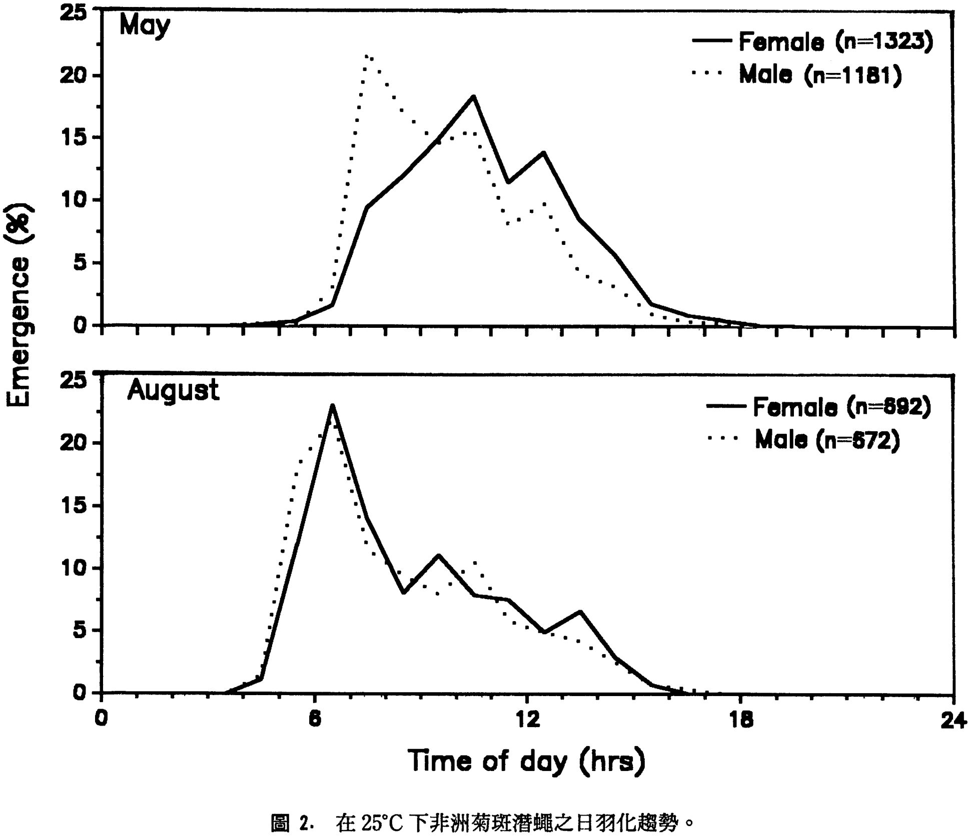

圖 2. 在 25°C 下非洲菊斑潛蠅之日羽化趨勢。
Fig. 2. Daily emergence trend of adult *Liriomyza trifolii* at 25°C.

交尾：交尾時雄蠅爬至雌蠅背上，彼此觸角相接觸，然後雄蠅後退，二者方向一致，尾端相接呈形。每次交尾時間爲 33 ± 2 分鐘(n= 30)。自上午 5 點至下午 5 點間均可見交尾個體，但 67

%之雌雄蠅集中於上午 5 點至 9 點間交尾(圖 3)。同時該蠅之交尾前期與其羽化時刻有關，如在 5 月時早上 6 點至 12 點間羽化之雌蠅，其中約 41%於當日上午 12 點至下午 5 點間交尾，交尾前期爲 6.5 ± 0.2 小時(n= 103)，另 59%之雌蠅則於次日上午 5 點至下午 1 點間交尾，交尾前期爲 21.5 ± 0.2 小時(n= 150)。而於早上 12 點至下午 4 點間羽化者，則必需延至次日上午 5 點至下午 1 點間才完成交尾，交尾前期爲 17.3 ± 0.1 小時(n= 138)(圖 4)。亦即在早上 12 點前羽化之成蠅，其中 2 %距羽化後 3 小時即可交尾，且羽化時刻愈早者當日完成交尾之比率愈高。如在早上 7 至 12 點間每小時內羽化之成蠅，其當日交尾比率各爲 63 、 55 、 34 、 36 及 18%。而於中午以後羽化之成蠅，不論羽化時刻之先後，其當日交尾率爲 0 。成蠅一生可行多次交尾。在僅供食蜂蜜時，雌、雄蠅之壽命各爲 4.8 ± 0.3 與 4.1 ± 0.1 天(圖 5)。其間該蠅各日齡之交尾率，在第一日齡時雖僅 26%，但至第二日齡時即達 99%，且第三至第六日齡與第七至第十一日齡時，每日仍可維持 72 ~ 82 與 46 ~ 57%之交尾率(表 3)。另外該蠅除第一日齡者 94%僅行 1 次交尾外，其它各日齡成蠅之每日交尾次數爲 2 、 3 及 4 次者，各佔 22.0 ± 1.8 、 2.1 ± 0.8 及 0.2 ± 0.1%(表 3)。

表 3. 在 25°C 下非洲菊斑潛蠅雌蠅各日齡之交尾率與交尾次數
Table 3. Daily mating rate and mating frequencies of female *Liriomyza trifolii* at 25°C

Age of adult (days)	No. observation		% Mating female	No. mating frequencies	% Distribution of mating frequencies			
	Female	Male			1	2	3	4
1	395	395	26	34	94	6	0	0
2	254	254	99	356	71	22	6	1
3	147	147	82	143	84	16	0	0
4	122	148	72	115	76	20	4	0
5	117	112	76	130	68	31	1	0
6	128	128	76	164	59	33	7	1
7	137	107	55	98	77	21	2	0
8	99	67	46	57	79	21	0	0
9	99	71	53	63	82	16	2	0
10	70	72	56	48	81	19	0	0
11	60	60	57	43	79	21	0	0

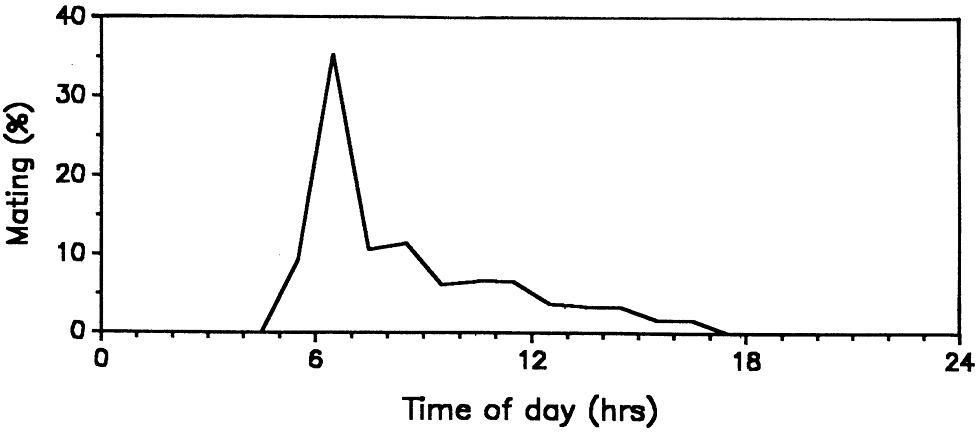

圖 3. 1995 年 5 月 18 至 20 日間在 25°C 下第二日齡非洲菊斑潛蠅成蠅之日交尾趨勢。
Fig. 3. Daily mating trend of two-day-old *Liriomyza trifolii* at 25°C during May 18—20, 1995.

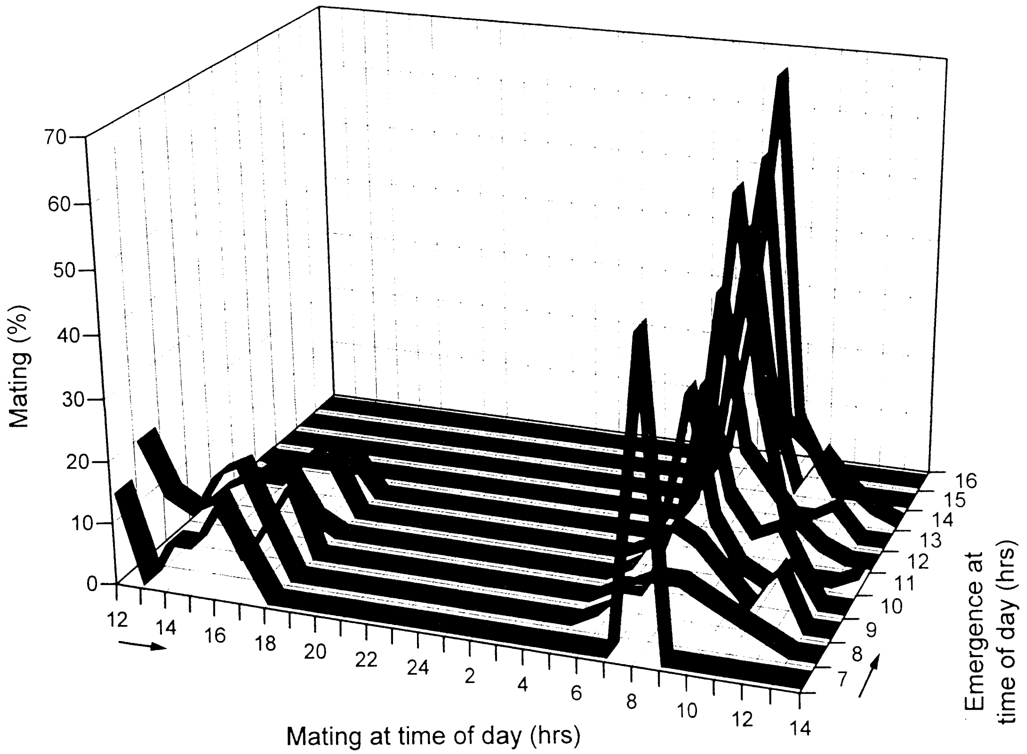

圖 4. 非洲菊斑潛蠅第一次交尾時刻與羽化時刻之關係。
Fig. 4. The relationship between the emergence time and the time of first mating for *Liriomyza trifolii.*

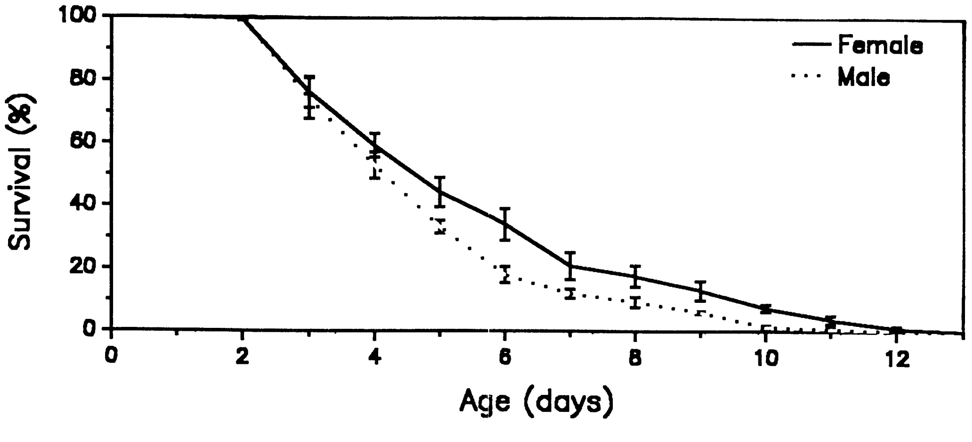

圖 5. 在 25°C 下供食蜂蜜時非洲菊斑潛蠅成蠅之日存活率曲線。
Fig. 5. Daily survival rate of adult *Liriomyza trifolii* provided with honey at 25°C.

產卵：非洲菊斑潛蠅在菜豆苗上僅產卵於眞葉上(32.3 ± 1.9 粒卵)，而不產卵於複葉，處理間呈顯著差異(t= 17.0237 ，df= 38)。產卵時利用產卵管刺破葉表皮，將卵產於其下，每產 1 個卵所需時間爲 24.5 ± 1.6 秒(n= 40)。產卵時刻爲早上 5 點至下午 7 點，且其間並無顯著之產卵高峰期(圖 6)。卵單粒散產。產卵孔可藉透光法辨識，其外形呈橢圓形(圖 1 -B)，長 0.29 ± 0.01 mm、寬 0.154± 0.002 mm(n= 20)。

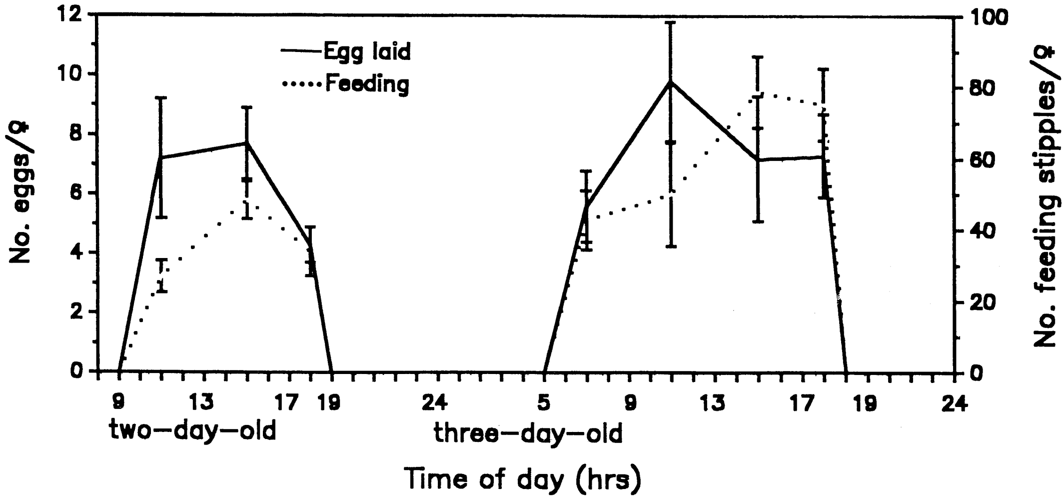

圖 6. 非洲菊斑潛蠅雌蠅在 25°C 與光周期 14 L：10 D(上午 5 點至下午 7 點間照光)下之日產卵與取食時刻。
Fig. 6. Daily oviposition and feeding trends of female *Liriomyza trifolii* at 25°C and 14 hrs of photophase set from 5:00 to 19:00 in growth chamber.

取食：非洲菊斑潛蠅羽化後除可取食水、蜂蜜外，雌蠅尙藉產卵管鑽刺寄主植物之葉表皮或91%之雌蠅(n= 45)待其產卵後，身體立即退後再以口器從其鑽刺處或產卵孔處取食寄主植物葉肉細胞之汁液，32%之雄蠅(n= 34)亦利用雌蠅之鑽刺處取食寄主植物汁液。若人爲干擾雌蠅在其產卵孔處之取食，則該卵之孵化率降爲 93.6%與正常卵之孵化率 99.0%間呈顯著差異(t= 2.8585 ，df= 16)。在菜豆苗上該蠅顯著偏好在眞葉上取食，即眞葉與複葉上之被害刻點數分別爲 132 ± 15 與 8 ± 3 個(t=8.2121、df= 38)。該蠅每次鑽孔取食約需 25.3 ± 1.2 秒，其中產卵管鑽刺需時 17.5 ± 1.0 秒，吸食汁液需時 7.3 ± 0.2 秒(n= 40)，而直接利用產卵孔吸食汁液者，吸食所需之時間爲 5.2 ± 0.4 秒(n= 29)。取食活動時刻爲早上 5 點至下午 7 點間。其中下午 1 點至 7 點間爲其取食高峰期(圖 6)。取食孔之分布呈聚集型。取食孔白色，近圓形(圖 1 -C)，長 0.36 ± 0.01 mm、寬 0.32 ± 0.01 mm(n= 20)。

雌蠅交尾次數與族群增長：由表 4 、5 及圖 7 之結果得知，非洲菊斑潛蠅雌蠅交尾與否雖顯著影響其繁殖力，但交尾次數之增加卻不影響其繁殖力。如僅交尾 1 次之雌蠅與雌蠅終生與雄蠅在一起且交尾 1 次以上者相較下，雌蠅之壽命、產卵期、取食期(取食寄主之日數)、產有活力之卵數、取食刻點數及子代成蠅數與雌性比間均無顯著差異。而雌蠅在未交尾時，不但壽命、產卵期、取食期、產卵數、取食刻點數較交尾者(1 或 1 次以上)各減少 9.4 ～ 9.7 天、11.1 ～ 12.1 天、8.8 ～ 9.7 天、477

粒卵或 496 ～ 567 粒有活力之卵及 1,518 ～ 2,112 個取食刻點數，產卵前期增加 2.7 ～ 2.9 天，且孵化率爲 0 ，致使其子代成蠅數(0 隻)與雌蠅僅交尾 1 次或 1 次以上之處理組間(377 ～ 411 隻)呈顯著差異。另由表 6 之結果亦得知，當非洲菊斑潛蠅雌蠅交尾 1 次與 1 次以上時，其雌性有活力卵數之每日內在增殖率各爲 0.2301 與 0.2329 。顯示非洲菊斑潛蠅祇要交尾 1 次即可維持其族群高增長率。

表 4. 在 25°C 下非洲菊斑潛蠅雌蠅之交尾次數對其壽命、產卵期及取食期之影響

Table 4. Influence of mating frequency of female *Liriomyza trifolii* on its longevity、oviposition and feeding period at 25°C

Mating frequency	N[z]	Longevity (days)[y]		Period (days)[y]					
		Female	Male	Pre-oviposition	Oviposition	Post-oviposition	Pre-feeding	Feeding	Post-feeding
0	6	14.3±2.2b	—	3.0±1.2a	7.0±1.2b	1.5±0.6a	1.0±0a	11.8±2.5b	1.3±0.6a
1	10	23.7±2.1a	—	0.3±0.2b	18.1±1.6a	2.4±0.5a	0.7±0.3ab	21.5±1.9a	1.1±0.3a
>1	9	24.0±1.8a	15.0±1.8	0.1±0.1b	19.1±1.5a	2.9±1.0a	0.1±0.1b	20.6±1.5a	2.2±0.9a

[z] N: number of observation.
[y] Means ($\bar{x}$±SEM) in each column followed by the same letter are not significantly different at 5% level by LSD.

表 5. 在 25°C 下非洲菊斑潛蠅雌蠅之交尾次數對其生殖力、取食及子代性比之影響

Table 5. Influence of mating frequency of female *Liriomyza trifolii* on its fecundity、feeding and progeny sex ratio at 25°C

Mating frequency	N[z]	Fecundity/♀[y]			Progeny sex ratio[y] (♀/♀+♂)	No. feeding stipples/♀[y]
		Eggs	Viable eggs[x]	Adults		
0	6	59±13	0b	0b	0b	1,179±219b
1	10	536±53	496±48a	377±35a	0.50±0.01a	3,291±332a
>1	9	—	567±44a	411±33a	0.53±0.02a	2,697±202a

[z] N: number of observation.
[y] Means ($\bar{x}$±SEM) in each column followed by the same letter are not significantly different at 5% level by LSD. Percentage of sex ratio data were transformed to arcsin $\sqrt{x}$ prior to ANOVA.
[x] Total number of 3rd-instar larvae.

表 6. 在 25°C 下非洲菊斑潛蠅雌蠅交尾 1 次與 1 次以上時之族群介量

Table 6. Population parameters of female *Liriomyza trifolii* for mating once or more at 25°C

Mating frequency	Parameters[z]			
	γ	λ	R_0	T
1	0.2301	1.2588	204	23.11
>1	0.2329	1.2623	188	22.47

[z] r : intrinsic rate of increase (day^{-1}); λ : finite rate of increase (day^{-1}); R_0 : net reproduction rate (viable female eggs/♀) ; T : mean generation time (days).

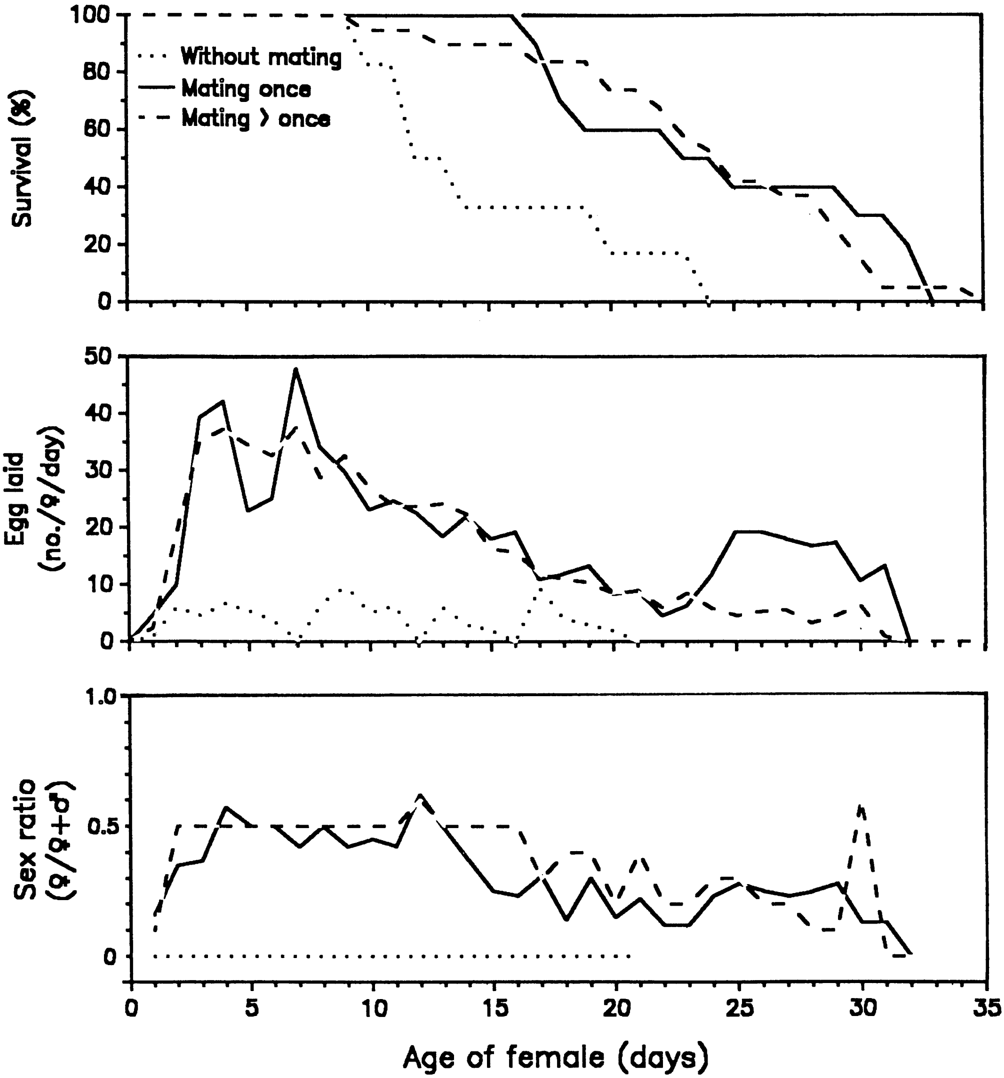

圖 7. 非洲菊斑潛蠅雌蠅在不同交尾次數時各日齡之存活率、產有活力之卵數及子代性比。
Fig. 7. Daily survival rate, fecundity (no. viable eggs) and progenies sex ratio of female *Liriomyza trifolii* at various mating frequencies.

二、溫度對非洲菊斑潛蠅發育之影響

由表 2 得知，在 10°～40°C 定溫下、非洲菊斑潛蠅卵發育至成蟲期之存活率受溫度之影響。該蠅發育之適溫帶爲 20° 至 35°C，其間卵發育至成蟲期之存活率達 74.5～81.6%，處理間無顯著差異。至於 10°～15°C 之低溫與 38°～40°C 之高溫則不適該蠅之發育。如在 40°C 時，蠅卵之孵化率爲 0；在 10°、12° 及 38°C 時，蠅卵之孵化率雖各高達 65.3、77.4 及 68.2%，但在彼等溫度時幼蟲之存活率卻僅各達 22.9、49.1 及 0 %；在 15°C 時，該蠅卵與幼蟲期之存活率雖高達 84.4 與 89.0%，但蛹期之存活率僅 19.5%，與其在適溫帶時之存活率呈顯著差異。另由表 1 得知，在 12°～35°C 時，非洲菊斑潛蠅卵至成蟲期之發育日數均隨溫度之升高而縮短，各處理組間呈顯著差異。同時由表 7 亦顯示該蠅各生長期之發育速率與溫度呈直線迴歸。卵、幼蟲、蛹及卵至成蟲期等各期之發育臨界低溫各爲 8.1、8.6、8.2 及 8.7°C，發育有效積溫各爲 50、79、161 及 294 日度。

表 7. 非洲菊斑潛蠅未成熟期之發育臨界低溫、發育有效積溫及發育速率

Table 7. The lower developmental thresholds (°C) and degree-day requirements (°C-days) and developing velocity for preimagial stage of *Liriomyza trifolii*

Stage	Lower developmental threshold[z] (x̄±SEM)	Degree-days[z] (x̄±SEM)	Regression equation	R^2
Egg	8.1±0.5	50±2	$\hat{y}=-0.1626+0.0201x$	0.99
Larva	8.6±0.9	79±8	$\hat{y}=-0.1104+0.0127x$	0.98
Pupa	8.2±1.2	161±23	$\hat{y}=-0.0513+0.0063x$	0.96
Egg to adult	8.7±0.9	294±32	$\hat{y}=-0.0292+0.0034x$	0.98

[z] Lower development thresholds and degree-days were estimated according to the methods of Campbell *et al.*(1974).

三、溫度對非洲菊斑潛蠅族群增長之影響

壽命：非洲菊斑潛蠅雌蠅與雄蠅在 12°～ 40°C、每日供應純蜂蜜與 1 株菜豆苗時，其壽命與溫度間各呈向下拋物線反應($\hat{y}$= 6.1416 + 1.3772 x－ 0.0349 x^2，R^2= 0.87 ；與$\hat{y}$= 1.2113 + 0.3284 x－ 0.0135 x^2，R^2= 0.85)。當溫度在 12°～ 25°C 時，雌、雄蠅之壽命顯著較長，各爲 16.0 ～ 23.4 天與 11.5 ～ 15.8 天；而當溫度在 30°～ 40°C 時，雌、雄蠅之壽命較短，各爲 7.2 ～ 11.4 天與 4.3 ～ 6.8 天。另當溫度相同時，雌蠅壽命均較雄蠅長(表 8)。

表 8. 非洲菊斑潛蠅在不同定溫下之壽命、產卵期及取食期

Table 8. Longevity、oviposition and feeding period of *Liriomyza trifolii* adults at various constant temperatures

Temp. (°C)	N[z]	Longevity (days)[y]		Period (days)[y]					
		Female	Male	Pre-oviposition	Oviposition	Post-oviposition	Pre-feeding	Feeding	Post-feeding
12	11	16.0±1.3b	14.5±1.2a	7.0±4.1b	1.5±0.3d	5.5±1.8a	4.5±1.3b	8.0±2.5c	3.4±1.1a
15	6	19.2±1.9ab	11.5±1.4ab	11.3±2.3a	3.3±1.5cd	1.5±0.3bc	10.3±1.2a	5.0±1.2c	1.8±0.5ab
20	7	21.3±1.6a	15.8±1.2a	1.3±0.7c	18.3±0.4a	0.4±0.2c	1.0±0.4c	19.9±1.4b	0.4±0.2b
25	19	23.4±1.5a	15.1±1.5a	0.1±0.1c	18.5±1.2a	3.0±0.7b	0.1±0.1c	20.6±1.2a	2.0±0.5ab
30	9	11.4±1.5c	5.1±0.9c	0.1±0.1c	9.0±1.3b	1.4±0.6bc	0.1±0.1c	9.4±1.3c	1.8±0.7ab
35	5	7.8±0.6c	6.8±1.0bc	0c	7.0±0.3bc	0.2±0.2c	0.6±0.2c	7.0±0.3c	0.2±0.2b
38	8	9.8±0.6c	5.8±0.5c	0c	7.9±0.8b	1.4±0.4bc	0.3±0.2c	8.8±0.6c	0.8±0.4b
40	6	7.2±0.5c	4.3±0.6c	0c	5.8±0.3bcd	0.7±0.2c	0c	6.2±0.5c	0.7±0.2b

[z] N: number of observation.
[y] Means (x̄±SEM) in each column followed by the same letter are not significantly different at 5% level by LSD.

產卵：由表 8 與 9 得知，非洲菊斑潛蠅產卵活動之適溫帶爲 15°～ 40°C，其中又以 20°～ 25°C 爲其最適溫度。該蠅終生產有活力之卵數與溫度(12°～ 40°C)間呈圓頂形拋物線反應($\hat{y}$=－ 1.0933 + 1.1421 x－ 2.1756 x^2，R^2= 0.91)。但在 20°～ 25°C 時，該蠅之產卵前期短(1.3 ～ 0.1 天)、產卵期長(18.3 ～ 18.5 天)、產卵數多(415 ～ 463 粒有活力之卵)。在 30°～ 35°C 時，該蠅之產卵前期雖短(0 ～ 0.1 天)，但產卵期(7 ～ 9 天)與產卵數(250 ～ 276 粒有活力之卵)亦相對較 20° 與 25°C 時顯著減少。在 38° 與 40°C 高溫時，該蠅之產卵前期(0 天)、產卵期(5.8 ～ 7.9 天)與在 30°～ 35°C 時無顯著差異，但產卵數卻銳減爲 125 ～ 127 粒卵或 9 ～ 96 粒有活力之卵。至於在 12° 與 15°C 低溫時，該蠅之產卵前期不但顯著延長爲7.0～11.3天，且其產卵期亦明顯減少爲1.5～3.3天，產卵數更減

至 2 粒卵與 1 ～ 20 粒有活力之卵。該蠅之產卵後期亦受溫度影響，在 12° 與 25°C 時，可長達 3 ～ 5.5天，而其它各處理組則 0.2 ～ 1.5 天不等。另由圖 8 得知，非洲菊斑潛蠅之日產卵型式依溫度而異。在 12°C 時，因低溫不適雌蠅產卵，其日產卵數變異大，日產卵趨勢不明顯。在 15°C 時，雌蠅除產卵日期明顯延遲至第十一天開始外，在其後 11 天之產卵期中，每日之產卵數(0.4 ～ 5.0 粒有活力之卵)變異亦大。在 20° 與 25°C 時，雌蠅雖各自第二與第一日齡開始產少數卵(3.1 與 2.5 粒有活力之卵)，但其產卵數隨後即明顯增加，且各經 21 與 13 天之產卵高峰期(每日各產 13.5 ～ 29.7 與 18.7 ～ 37.5 粒有活力之卵)後才緩慢下降。至於在 30° 與 35°C 時，各處理組雌蠅之日產卵型式相似。雌蠅除第一日齡時即產 6 ～ 9.9 粒有活力之卵外，至第二日齡時之產卵數已迅速各增加爲 31.7 與 67.4 粒有活力之卵，但其產卵盛期不長，僅各維持 7 與 4 天即下降。而在 38° 與 40°C 時，雌蠅之日產卵型式雖與 35°C 時相似，但其每日之產卵數已大幅銳減。

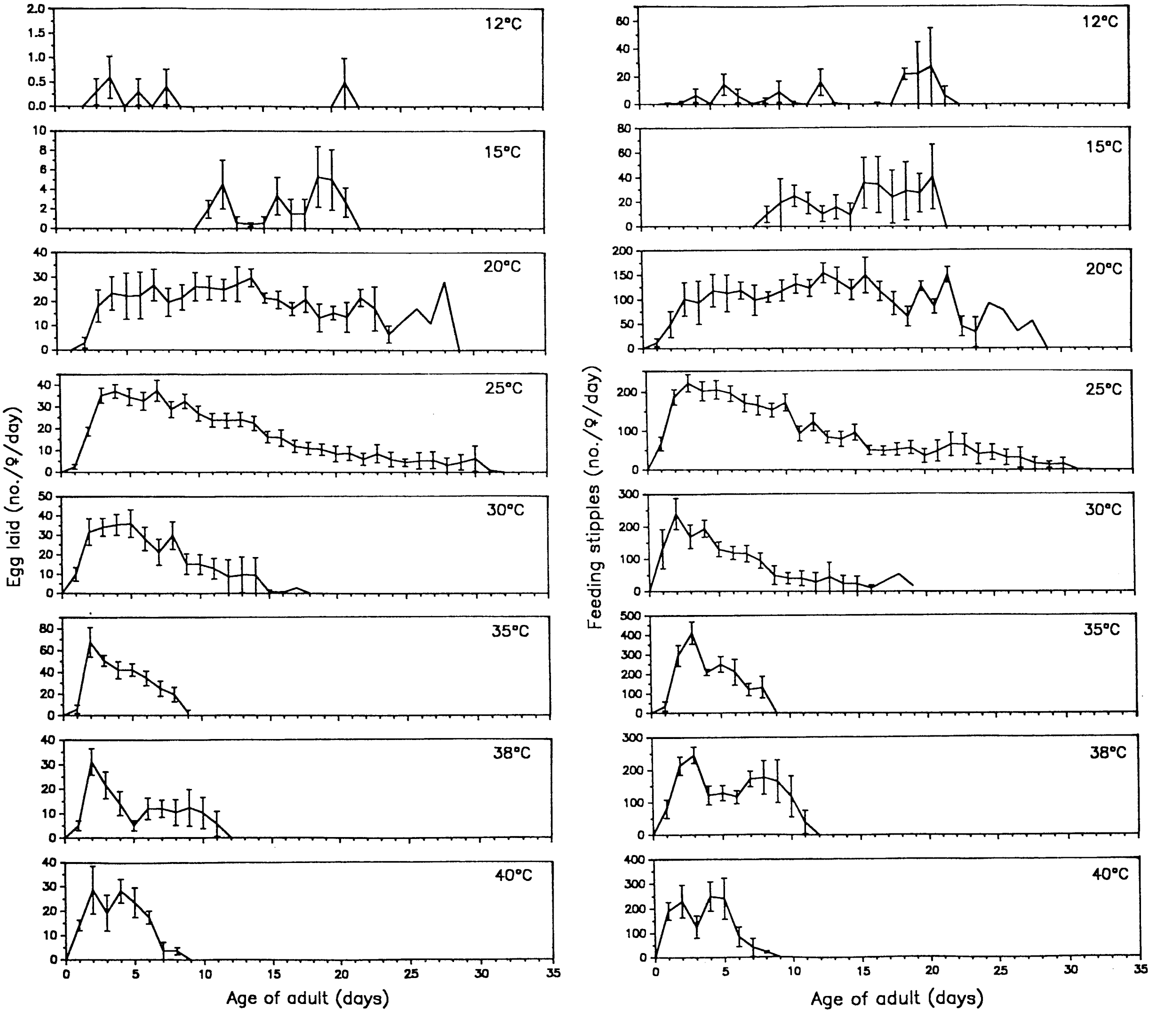

圖 8. 非洲菊斑潛蠅雌蠅在不同定溫下各日齡之生殖力與取食刻點數。

Fig. 8. Daily fecundity and feeding stipples of female *Liriomyza trifolii* at various constant temperatures. Data for 15°—35°C obtained from viable eggs, data for 12°, 38° and 40°C from actual eggs deposited.

取食：由表 8 與 9 得知，非洲菊斑潛蠅成蠅能夠取食之溫度帶爲 12°～ 40°C，其中又以 20° 與 25°C 時之取食最爲頻繁。同時該蠅之取食刻點數與溫度（12°～ 40°C）間呈圓頂形拋物線反應（$\hat{y} = -4.3024 + 4.5696x - 8.0889x^2$。$R^2 = 0.83$）。另由圖 8 得知，非洲菊斑潛蠅之日取食型式不但與溫度有關，且在 15°～ 40°C 間、當溫度相同時，該蠅之日取食型式與其產卵型式相似。但在 12°C 時，由於低溫，雌蠅之日取食活動非持續性發生且變異大，致使其日取食型式呈波狀起伏。

產卵與取食之比值：由表 9 得知，非洲菊斑潛蠅之產卵與取食之比值與溫度（12 ～ 40°C）間呈圓頂形拋物線反應（$\hat{y} = -0.2992 + 0.0368x - 0.0007x^2$，$R^2 = 0.91$）。當溫度在 20°～ 35°C 時，該蠅之產卵與取食之比值較高爲 0.18 ～ 0.20 ，而當溫度爲 12°、 15°、 38° 及 40°C 時，該值則顯著降低各爲 0.05 、 0.08 、 0.08 及 0.12 。

表 9. 非洲菊斑潛蠅在不同定溫下之繁殖潛能與取食刻點數

Table 9. Reproductive potential and feeding stipples of *Liriomyza trifolii* at various constant temperatures

Temp. (°C)	N[z]	Fecundity/♀[y]		Progeny sex ratio[y] (♀/♀+♂)	No. feeding stipples/♀[y]	Ratio[y,w]
		Eggs	Viable eggs[x]			
12	11	2±1	1± 1c	0.56±0.06a	71± 23d	0.05±0.04c
15	6	—	20±10c	0.56±0.08a	224± 98d	0.08±0.03c
20	7	—	415±57a	0.48±0.02a	2,213±284ab	0.20±0.01a
25	19	—	463±36a	0.52±0.01a	2,580±158a	0.18±0.01a
30	9	—	250±37b	0.49±0.01a	1,309±122c	0.19±0.02a
35	5	—	276±14b	0.49±0.03a	1,590± 94bc	0.18±0.02ab
38	8	127±20	96±19c	0.48±0.01a	1,558±165c	0.08±0.01c
40	6	125±19	9± 2c	0.31±0.12b	1,194±157c	0.12±0.01bc

z N: number of observation.
y Means (x̄±SEM) in each column followed by the same letter are not significantly different at 5% level by LSD. Percentage of sex ratio were transformed to arcsin $\sqrt{x}$ prior to ANOVA.
x Total number of 3rd-instar larvae.
w Oviposition punctures/feeding stipples.

雌性比：由表 9 得知，當溫度在 12°～ 38°C 時，非洲菊斑潛蠅之子代雌性比均各在 0.48 ～ 0.56 間，處理間無顯著差異。而溫度在 40°C 時，由於高溫不適該蠅卵之發育，致使其有活力之卵數銳減，間接降低其子代之雌性比爲 0.31 。

齡別生命表：由表 10 得知，在 12°～35°C 間，非洲菊斑潛蠅之族群增長隨溫度之上升而增加，如

表 10. 非洲菊斑潛蠅在不同定溫下之族群介量

Table 10. Population parameters for *Liriomyza trifolii* at various contant temperatures

Temp. (°C)	N[z]	Parameters[y]							
		Viable female eggs[x]				Female adults			
		γ	λ	R_0	T	γ	λ	R_0	T
12	11	−0.0030	0.9970	0.8	94.78	−0.0088	0.9913	0.4	93.52
15	6	0.0051	1.0057	1.6	83.33	−0.0004	0.9995	1.0	83.78
20	7	0.1684	1.1834	151.7	29.82	0.1562	1.1691	108.9	30.00
25	19	0.2329	1.2622	187.5	22.47	0.2183	1.2439	136.1	22.51
30	9	0.2722	1.3128	93.7	16.68	0.2529	1.2877	66.1	16.57
35	5	0.3236	1.3821	101.4	14.27	0.3053	1.3571	82.6	14.46

z N : number of observation.
y r : intrinsic rate of increase (day^{-1}); λ: finite rate of increase (day^{-1}); R_0: net reproduction rate (viable female eggs or female adults/♀) ; T: mean generation time (days).
x Total number of 3rd-instar larvae.

其雌性有活力卵數之每日內在增殖率各為－0.0030、0.0051、0.1684、0.2329、0.2722及0.3236。雌性成蠅之每日內在增殖率各為－0.0088、－0.0004、0.1562、0.2183、0.2529及0.3053。顯示35°C為該蠅族群增長最快之溫度。但由該蠅之淨增殖值觀之，則顯示25°C為該蠅族群增長之最適溫度(圖9)。

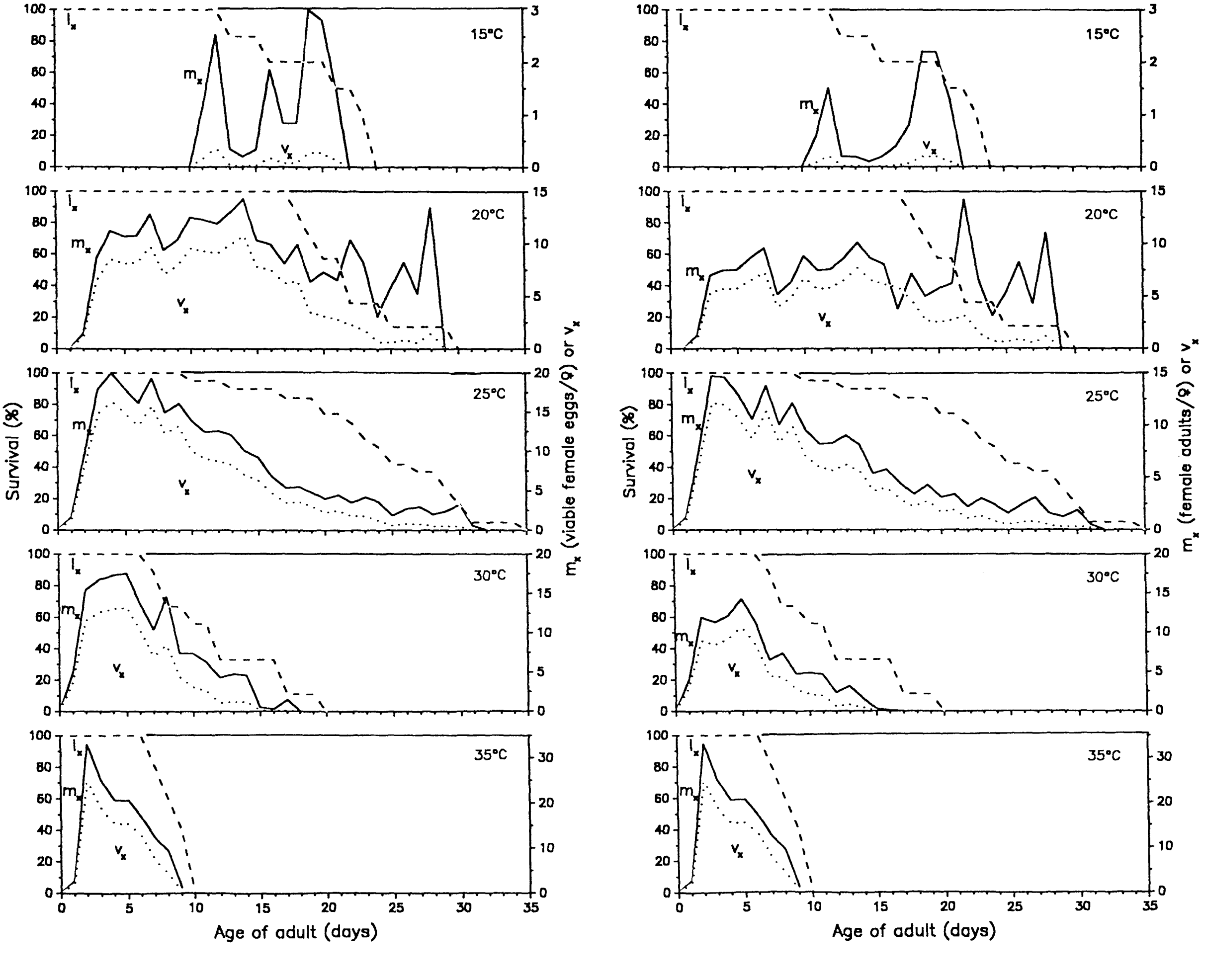

圖 9. 非洲菊斑潛蠅在不同定溫下之齡別存活率(l_x)、繁殖率(m_x)、及淨增殖值($v_x=l_xm_x$)。
Fig. 9. Age-specific survival rate (l_x)、fecundity rate (m_x) and distribution of net maternity value ($v_x=l_xm_x$) of *Liriomyza trifolii* at various constant temperatures.

四、低溫對非洲菊斑潛蠅蛹貯存之影響

由表11得知，非洲菊斑潛蠅之蛹不耐低溫貯存。如將第一日齡之蠅蛹在8°、12°及15°C下貯存1星期後，其羽化率各為0、66.7及66.7%，與對照組間呈顯著差異。若蠅蛹在12°與15°C下貯存3～4星期後，其羽化率更降低至50.0～58.3與46.7～56.7%。

表 11. 非洲菊斑潛蠅蛹經不同溫度貯存 0 至 4 週後在 25°C 下之羽化率

Table 11. Emergence rate of adult *Liriomyza trifolii* at 25°C after storing pupae at various temperatures for 1 to 4 weeks

Duration (weeks)	% Emergence[z,y]		
	8°C	12°C	15°C
0(ck)	83.3±1.7a	81.7±1.7a	85.0±2.9a
1	0b	66.7±3.3b	66.7±4.4b
2	0b	66.7±1.7b	63.3±1.7b
3	0b	58.3±4.4bc	56.7±4.4bc
4	0b	50.0±7.6c	46.7±4.4c

z Means ($\bar{x}$±SEM) in each column followed by the same letter are not significantly different at 5% level by LSD. Percentage of emergence were transformed to arcsin $\sqrt{x}$ prior to ANOVA.

y Twenty pupae per treatment with 3 replications.

討 論

據 Charlton and Allen 之觀察，非洲菊斑潛蠅成蠅之羽化、產卵、取食及老熟幼蟲之鑽出葉面化蛹等行爲，係屬晝行性(diurnal)，且其盛期在中午之前[10]。而本試驗結果，除印證上述該蠅之日律性外，尙得知該蠅之羽化時刻及交尾前期均與黎明時間有關(圖 2 、4)。黎明時間早時，該蠅羽化時刻不但提早且較集中，同時其交尾前期亦可明顯縮短。但有關非洲菊斑潛蠅羽化時之臨界光度仍待探明。

Oatman and Michelbacher 認爲斑潛蠅 *Liriomyza pictella* (Thomson) 雌蠅之重複交尾爲其產下最多受精卵之必要條件[17]。本試驗之結果，雖顯示非洲菊斑潛蠅有多次交尾之習性(表 3)，但亦證實該雌蠅僅交尾 1 次時，其繁殖子代成蠅數、雌性比等與雌蠅交尾 1 次以上者間無顯著差異(表 3)。因而顯示非洲菊斑潛蠅雌蠅僅需交尾 1 次即可維持其族群之高增殖率(表 6)。至於該蠅多次交尾之意義，推測或與增加其基因之歧異度有關。

非洲菊斑潛蠅由其日產卵型式得知，卵形成方式屬應變式產卵(synovigeny)策略。至於其日平均產卵數與取食數變異値較大之原因，係雌蠅在其一生之產卵期或取食期中，非周期性出現當某一天或連續數天之產卵或取食數突然增多時，隔天其產卵數或取食數即迅速下降，甚至發生不產卵或不取食之現象。

非洲菊斑潛蠅雌蠅產卵數之估算有兩種方法，一爲應用酸性品紅溶液(lactophenol-acid fuchsin solution)之染色法計數其在寄主葉內所產下之卵數[22]，另一爲目視計數在寄主葉內之初孵化幼蟲數以估算其產下之有活力之卵數[18]。其中前者因需額外花費試驗藥品費，當寄主葉片取樣數大時，卵數調查受限[22]；而後者不但方法較快速簡便，且不影響該蠅子代生物性之觀察。由本試驗結果得知，由於菜豆葉片較薄，雖可直接藉鏡檢法計數菜豆葉內非洲菊斑潛蠅之卵數，但當卵數過多時，計數工作仍費時費力。同時在室內 20°～ 35°C 時，非洲菊斑潛蠅卵之孵化率與幼蟲之存活率均高達 94 ～ 99% 與 90 ～ 98%(表 2)。因而顯示在室內不但可利用菜豆葉內非洲菊斑潛蠅之第三齡幼蟲數進行該蠅生殖力中產卵數之估算，且此法較計數初齡幼蟲數又更爲醒目易行。

非洲菊斑潛蠅幼蟲之危害，以第三齡幼蟲居最。在菊花上其食痕率爲第一與第二齡幼蟲之 9.4 與 4.5 倍[20]。本試驗雖未利用電腦軟體(high Pad digitizing software)準確比較該蠅各齡幼蟲在菜豆苗上之食痕率，但僅由其食痕長度觀之，第三齡幼蟲即爲第一與第二齡幼蟲之 9.0 與 4.7 倍，而由幼蟲之食痕寬度亦得知，該蠅第三齡幼蟲隨其發育之增長，其對寄主植物之危害亦增大(圖 1 -F)。另據錢景秦、古琇芷未發表之資料得知，在臺灣非洲菊斑潛蠅本地寄生蜂中之異角釉小蜂、華釉小蜂

(*Neochrysocharis formosa* (Westwood))、岡崎釉小蜂(*Chrysonotomyia okazakii* (Kamijo))及底比斯釉小蜂(*Chrysoncharis pentheus* (Walker))，均對該蠅第三齡幼蟲具產卵偏好性，且彼等之寄生方式均屬非共育寄生性(idiobiont)策略，亦即寄主一被寄生即失去取食能力，並於數小時內死亡。因而顯示彼等寄生蜂對非洲菊斑潛蠅第三齡幼蟲所造成之危害具有強勢之抑制力。

斑潛蠅 *Liriomyza munda* Frick 幼蟲之危害與寄主植物之種類與品系間關係密切，其中萊豆(Henderson bush lima bean)之眞葉最適該蠅之生長發育，而不同品系之番茄與菊花則對該蠅呈現程度不一之抗生作用[28]。在非洲菊斑潛蠅方面，本試驗結果得知，該蠅第一、二及三齡幼蟲在菜豆眞葉上之食痕長度為 Parrella and Bethke[20] 在菊花上測得該蠅各齡幼蟲食痕長度之 2.1 、 2.1 及 4.6 倍，因而顯示非洲菊斑潛蠅在菜豆眞葉上因食痕所造成之危害較菊花為重。

非洲菊斑潛蠅對寄主植物之危害，除幼蟲之潛食葉肉組織外，雌成蠅在取食與產卵行為中亦對葉面造成不同大小之白色刻點，影響寄主植物之光合作用。Spencer 認為雌成蠅之取食有 3 種作用，分別為確認寄主植物、自寄主植物中獲得蛋白質供其卵之形成、及取食可利用之碳水化合物[25]。而 Hussey and Gurney 亦謂可藉有活力之卵數與取食孔數之比值，做為潛蠅類對寄主植物偏好性之指標[12]。但其中有關成蠅之取食孔數，因學者間對其認知上之差距，以致造成文獻上之混亂[19]。本試驗證實非洲菊斑潛蠅雌蠅當取食寄主植物葉液時，不僅從其取食孔獲得，亦可自其產卵孔處取得[6]。因而豆葉上之所有葉面被害刻點數，可視為該蠅之取食刻點數。另方面亦發現，雌蠅在產卵後立即在其產卵孔處之吸食行為，實可提高卵之孵化。同時又因該蠅之卵形成方式屬應變式產卵策略，因此其卵在發育中並不增大，因而此點與 Dimetry 所謂可藉寄主植物之汁液供蠅卵在發育中增大一事[11]，有極大差異。

非洲菊斑潛蠅為一雜食性昆蟲，寄主種類超過 400 種[19]，但其繁殖力受寄主植物種類、葉品質、溫度及食物等之影響[16]。因而為達該蠅繁殖方法之建立，必先瞭解彼等因子與該蠅繁殖之關係。前人研究顯示，豆類較菊花適合該蠅之發育與存活[10]。而菊花(279 ～ 298 粒卵／雌)與芹菜(212 粒卵／雌)又較蕃茄(40 粒卵／雌)適合該蠅之生殖[18,24]。在菊花與芹菜上，該蠅族群增長最大之溫度，推測為 25° 與 30°C，而 15°C 時，族群則幾乎停止增長[16]。另當雌蠅供食蜂蜜後，其壽命(22.7 天)與產卵數(439 粒卵)較未供食者可提高 3 倍[10]。而由本試驗結果得知，在室內菜豆苗上每天供食純蜂蜜情況下，非洲菊斑潛蠅確具高繁殖力(表 9 、 10 及圖 9)。在 20°～ 35°C 間，其雌性有活力卵數之每日內在增殖率(γ)與終極增殖率(λ)均隨溫度之上升而增加，各達 0.1684 至 0.3236 與 1.1834 至 1.3821，而 15°C 時，族群則幾乎停止增長。但若就族群之淨增殖率而言，其中以 20° 與 25°C 最適該蠅之生殖(152 與 188 粒雌性有活力之卵／雌)。另外在臺灣田間菜豆成株之複葉上雖可發現該蠅之危害[1]。但本試驗結果顯示，該蠅在菜豆苗上卻對眞葉與複葉間具極顯著之產卵與取食偏好性，且完全不產卵於複葉上。因而在室內繁殖非洲菊斑潛蠅時，其最適條件，為在 25°C、每日供食純蜂蜜情況下，以 15 ～ 20 cm 莖高之菜豆苗供該蠅產卵。

非洲菊斑潛蠅對黃色具偏好性。臺灣在 1988 至 1989 年曾成功地利用黃色黏板進行該蠅侵入後之區域性與全省性之發生偵測調查[3]。至於黃色黏板在非洲菊斑潛蠅防治效果方面，由於黃色黏板之誘蟲距離小於 10 m(甚或小於 2 m)，且在密閉非洲菊園設施內，每隔 2 m 放置一塊黃色黏板之情形下，第一至第四日內經過死亡率校正後，雌蠅之誘捕回收率各為 4.6 、 5.5 、 5.5 及 8.2%，雄蠅之誘捕回收率各為 12.6 、 3.9 、 4.6 及 7 %[2]，顯示就以雌、雄性之誘捕率而言，雄蠅較高，同時在 80.7% 之誘捕雌蠅中，即第二至第四日間被誘捕之雌蠅，或許均已具產卵經驗。而本試驗亦證實非洲菊斑潛蠅之交尾前期，雖受其羽化時刻之影響，但至羽化後 21.5 小時內，其交尾率即達 99%，且其一生中之交尾次數，估測少者 4 ～ 5 次，多則可能在 20 次以上。同時雌蠅經交尾 1 次後，不但終生產受精卵，且其壽命、生殖力、取食刻點數、子代雌性比及族群增長等均不受影響，而其產卵前期亦僅 0.3

天(表 4 、 5 、 6)。因而顯示，欲單獨利用黃色黏板誘集雌蠅或雄蠅之方法以降低非洲菊斑潛蠅之密度，實有其限制。另寄生蜂對黃色亦具偏好性，當田間設置大量黃色黏板時，亦可能影響寄生蜂對非洲菊斑潛蠅之抑制效果。

評估寄生蜂對害蟲之抑制力與有效性之前，必先瞭解害蟲與寄生蜂在各不同溫度下彼此之內在增殖率。Minkenberg and Lenteren 認爲有效寄生蜂之內在增殖率除應較其寄主之內在增殖率大或相等外，另當寄生蜂有取食寄主之現象時，尙需將其取食致死寄主之能力併入，供做寄生蜂抑制寄主能力之整體考量[16]。由本試驗結果得知，非洲菊斑潛蠅之繁殖力雖強，在 25°、 30°及 35°C時，其雌性成蠅每日之內在增殖率各爲 0.2183 、 0.2529 及 0.3053 (表 10)。但由其兩種寄生蜂一華釉小蜂與異角釉小蜂在 25°C、每日供應 40 隻寄主幼蟲時，其雌性成蜂每日之內在增殖率各爲 0.2038 與 0.2569，及兩種寄生蜂之取食寄主能力又各爲其寄生能力之 0.8 與 1.4 倍(錢景秦、古琇芷，未發表資料)，因而顯示此兩種寄生蜂對非洲菊斑潛蠅極具防治潛能。

於 1988 年在臺中大坑地區發生的非洲菊斑潛蠅，因缺少先前該蠅在臺灣之發生記錄，故推測係一國外新侵入種[1,27]，後經分類學上之硏究，認爲比較可能爲來自荷蘭一抗藥性品系侵入[5]。但由本試驗結果得知，臺中地區該蠅在菜豆(*P. vulgaris* var. *communis*)上其卵至成蟲期之估測發育臨界低溫(8.7°C)與實測致死高溫(38°C)及產卵時之實測臨界低溫(≦ 12°C)與高溫(> 40°C)等值(表 2 、 7 、 9)，均較 Charlton and Allen[10]、Parrella[18]及 Vercambre and Thiery[26] 所報導在菜豆(*P. vulgaris*)上該蠅卵至成蟲期之發育臨界低溫(8.4°C)與致死高溫(35°C)及在菊花上之產卵臨界低溫(10°C)與高溫(近 40°C)等值要高。其間或受寄主植物種類與品系之影響，但其主因或係臺中地區非洲菊上之非洲菊斑潛蠅爲適應本地簡易溫室內之溫度(夏季高達 40°C 以上)，其已發展出對高溫較具忍受性之品系。

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Morphology, Life History and Reproductive Ability of *Liriomyza trifolii*[1]

Ching-Chin Chien and Hsiu-Chih Ku[2]

Summary

Morphology, life history, and effect of mating frequence and temperature on the population growth rate of the leafminer, *Liriomyza trifolii* (Burgess) were examined in the laboratory with field bean, *Phaseolus vulgaris* var. *communis* Aeschers as host plant. Results indicated that emergence, oviposition and feeding of adults as well as larvae emerging from leaves was in diurnal pattern. The mating rate reached 99% on the 2nd day. Males and females mated more than once (4—20's times). At 25°C, unmated females could lay fewer infertile eggs. The intrinsic rate of increase (γ) for female of mating once or more than once was 0.2301 or 0.2329/day. The female fed from all punctures regardless some for oviposition. The egg hatching rate could be increased if female fed on oviposition site. Female significantly preferred laying eggs and feeding on primary leaf but completely did not lay eggs on trifoliate leaf of field bean. Egg production strategy belongs to synovigeny, and the egg size did not increase in development. The number of the third larva within the mesophyll could be used to estimate the oviposition of leafminer between 20°—35°C due to the high survival rates of egg and larval stages. The length of mine increased with the growing instar of which the third instar was ca. 9.0- and 4.7-fold longer than that of the first and second instars, respectively. The observed lowest and highest temperature for oviposition were at 12° and 40 °C. The lower developmental threshold was estimated to be 8.7°C from egg to adult. The upper lethal limit temperature for development was 38°C. The maximum population growth of *L. trifolii* can be expected at 35°C (γ=0.3236/day), while almost no increase at 15°C (γ =0.0051/day) when honey is provided. The optimum temperature for reproduction was between 20°−25°C (R_0=152−188 viable female eggs/female). At 25°C, its development period and survival rate from egg to adult was 16.6 days (egg stage 3.3 days, larval stage 4.6 days, pupa stage 8.6 days) and 81.6%, respectively. The optimal conditions for rearing *L. trifolii* were using 15—20 cm tall field bean for adult infestation and daily provided with honey at 25°C.

Key words: *Liriomyza trifolii,* Life history, Population growth, Field bean , Temperature.

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1. Contribution No. 1832 from the Taiwan Agricultural Research Institute.
2. Associate Entomologist and project assistant of Department of Applied Zoology, TARI, Wufeng, Taichung, Taiwan, ROC.

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Management of Resistant Diamondback Moth and Other Crucifer Pests in Taiwan[1]

Edward Yun Cheng[2], Chiu-Nan Chen[3] and Ching-Hua Kao[2]

Abstract : Resistant diamondback moth (*Plutella xylostella*, DBM) was managed by insecticide alternation of organophosphorus insecticides, *Bacillus thuringiensis* (B.t.) and cartap to avoid cross resistance. The rationale of insecticide selection was discussed in detail. Other pests were also considered in the design of the implementation program. In this implementation program, several counter measures were taken for removing the constraints, including pesticide dependency, misuse of pesticide, marketing competition, etc. After 3-year test (1990—93), the evaluation indicated a 16.7% increase in crop income, 35% saving in insecticide expenditure, lengthening the spray interval by 2.1 days (from 5.7 days to 7.8 days), and 1.6 insecticides reduction (from 4.4 to 2.8 insecticides) per tank mixture.

Key words : Diamondback moth (*Plutella xylostella*), Resistance management program, Insecticide alternation, Nucleus farmers

INTRODUCTION

A serious insecticide resistance in the diamondback moth (*Plutella xylostella*, DBM) occurred in 1970s. It not only resulted in failure of recommended pest control measures but also caused many pesticide related problems such as (1) aimless mixing of pesticides, (2) cost increase in vegetable protection, and (3) residual problem from overuse of pesticides, etc. In order to solve these problems, Taiwan Agricultural Research Institute (TARI) initiated a DBM research program in 1980, which consisted of the following parts :

1. Developing a survey method for detecting resistance.
2. Conducting a general survey of resistance in DBM.
3. Elucidating the characters of insecticide resistance.
4. Examining the cross resistance amongst insecticides.
5. Designing a strategy for managing resistant DBM.
6. Implementing the resistance management program (RMP).

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1. Contribution No. 1834 from Taiwan Agricultural Research Institute.
2. Senior entomologist and assistant entomologist, respectively, Department of Applied Zoology, TARI, 189 Chung-cheng Rd., Wufeng, Taichung, Taiwan.
3. Chief of Plant Protection Division, Council of Agriculture, 37 Nan-hai Rd., Taipei 100, Taiwan.

The first two parts were completed and reported in 1981[3,4], and update reports of resistance survey were followed[8,9,12,23]. The third and fourth parts were completed in 1987, which laid the fundation for the insecticide alternation strategy as proposed in 1987[5]. Later, the Council of Agriculture (COA) assigned TARI to coordinate TACTRI (Taiwan Agricultural Chemical and Toxic-substances Research Institute) and AVRDC (Asian Vegetable Research and Development Center) on three projects below.

1. Biological control of resistant DBM led by Dr. N. S. Talekar of AVRDC.
2. The ecology and population dynamics of DBM by Mr. W. Y. Su of TACTRI.
3. Implementation of insecticide alternation strategy for managing the resistant DBM, conducted by Dr. E. Y. Cheng of TARI.

For the first part of the program, Dr. Talekar of the AVRDC reported that the DBM on highland crucifers had been successfully brought to a level below the economic threshold by an effective natural enemy, *Diadegma semiclausum* with supplementary sprays of microbial insecticide, *Bacillus thuringiensis* (B.t.)[36]. Unfortunately, the same practice was not successful on lowland crucifers, and more complicated pesticide application on lowland was suggested as the reason for failure.

The second part of the program i.e., ecology and cultural control strategy of DBM, was reported[13] in 1993.

In the third part of the program, TARI had invited 6 farmers from two farmer associations to test the insecticide alternation strategy in September of 1990. The preliminary trial received favorable comments[10], and the project was expanded to 68 farmers from 8 farmer associations in the following year. The evaluation of the second year's result prompted COA and PDAF (Department of Agriculture and Forestry, Taiwan Provincial Government) to accept the strategy as a practical way to manage the resistant DBM. In 1993, the program was officially adopted for extension by 29 farmer associations with 192 farmers participated.

After confirming the farmer's acceptance of resistant DBM management strategy, we now report the design, structure and function of this implementation program.

RATIONALES OF DESIGNING THE RESISTANCE MANAGEMENT STRATEGY FOR DBM

We believe that with a clear knowledge of resistance and cross resistance, farmers can be educated to select suitable insecticide to reduce or avoid the resistance, and then a resistance management program based on the alternation of insecticides with minimal cross resistance can be implemented. The following outlines are the characters of DBM resistance (Table 1) which provide rationales for selecting insecticides.

1. Resistance characters of DBM

(1) MFO-involved resistance I : Carbamate insecticides

Table 1. Resistance and cross resistance characters of DBM to different categories of insecticides

Resistance profile	OPs	Carbaryl methomyl and propoxur	Carbofuran	Synthetic pyrethroids	IGRs	Cartap	*Bacillus thuringiensis*
Resistance stability	stable to unstable	stable	unstable	unstable	unstable	stable	unstable
Resistance amplitude	medium	high	high	high	high	medium	medium
Resistance mechanism involved	multifactorial	monofactorial	monofactorial	monofactorial	monofactorial	monofactorial (proposed)	monofactorial (proposed)
Responsible biochemical entities	insensitive AChE GSTase carboxyl esterase esterase	MFOn	MFOc	MFOs	MFOi	broad spectrum esterase (proposed)	change of the receptors in the epithelium of midgut
Practical synergist	TDP	PB	PB	PB	PB	—	—
Genetic expression	intermediate	dominant	intermediate	intermediate	recessive	additive (proposed)	
Comments for field usage	OPs with unstable resistance are useful in the insecticide alternation program	Not recommended	Not recommended for the fast growing crucifer, occasionally use in the early growing stage to avoid residue problem	Strongly recommended for crucifer to control pests other than DBM	Can be used in a mosaic or alternation spraying program	Recommended for the insecticide alternation except in season with heavy precipitation	Recommended for the insecticide alternation, especially in the near-harvest period for safety consideration

AChE : acetylcholinesterase
MFOn : natural inherent MFO
MFOs : synthetic pyrethroid-induced MFO
TDP : 2-(3, 5-dichlorophenyl)-2-(2, 2, 2-trichloroethyl)oxirane
PB : piperonyl butoxide
GSTase : glutathione-S-transferase
MFOc : carbofuran-induced MFO
MFOi : IGR-induced MFO

Mixed function oxidases (MFOs) play a major role in detoxifying carbamates in many insects[1,2], and MFOs are also responsible for the poor performance of carbamates in DBM[14]. Carbamates are not very effective even against susceptible DBM. For instance, carbaryl, propoxur or methomyl were the least toxic material among 15 insecticides tested against a French susceptible strain[26]; and carbaryl and methomyl were also ineffective on Taiwan susceptible IL strain[18]. Despite that, as a member of the carbamate group, carbofuran is exceptionally effective and is not affected by the MFO which detoxify carbaryl and methomyl[14,18]. However, when DBM was subjected to carbofuran selection, a new MFO for resistance to carbofuran developed readily within several generations but with minimal cross effect to other insecticides [18,37].

There is no cross resistance between pyrethroids and carbamates. In other words, the MFOs which oxidize carbamates in DBM are not capable of detoxifying pyrethroids. This is based on the following premises : (1) synthetic pyrethroids provided excellent DBM control when it was first introduced, despite that carbaryl or methomyl had been ineffective for years ; (2) there was no cross resistance to pyrethroids in the carbofuran-selected resistant strain[18] ; (3) in the susceptible IL strain, piperonyl butoxide synergized carbaryl at ca. 47-fold but had no effect on carbofuran or three synthetic pyrethroids tested[14].

In summary, carbamates are not recommended for DBM control because they induce resistance readily, cause resurgence problem[27], and are higly toxic to non-target organisms.

(2) MFO-involved resistance II : Synthetic pyrethroids

Cross resistance within the synthetic pyrethroid (SP) group is commonly occurred in DBM[8] i.e., the resistance induced by one synthetic pyrethroid can extend to other members of the group[18,26].

DBM will lose the SP-resistance upon removal of the selection pressure. The rate of loss varies in different strains[14,35]. However, strong resistance will reappear readily when SP-selection resume. It is assumed that MFOs play a major role in the SP-resistance since most of the resistance is inhibited by the piperonyl butoxide (PB). A residual PB-insensitive resistance still existed and depended on which synthetic pyrethroid was tested[35]. Liu et al.[25,26] reported a 2-fold synergism by esterase inhibitors for permethrin in DBM. In another lepidopterous pest, *Spodoptera littoralis*, the increased esterase activity was strongly linked to the development of SP-resistance[31].

(3) MFO-involved resistance III : Insect growth regulators (IGRs)

In 1987, benzoylphenyl urea (BPU)-type IGRs were introduced for DBM control in Taiwan. Since then, the IGR resistance has been a great concern. The MFO of susceptible DBM possess an inherited metabolic activity toward diflubenzuron and triflumuron[15], and a mild teflubenzuron resistance was induced in DBM by laboratory selection [29]. The MFO degradation was considered as the resistance mechanism for IGRs in houseflies[30] and DBM[29]. In Taiwan, the sensitivities (LD_{50}) of DBM toward chlorfluazuron and teflubenzuron were below 10 mg/l before their registration in 1987[5].

However, the susceptibility (LD_{50}) of DBM to chlorfluazuron in Lu-chu area had increased from 3.8 mg/l in March to 924 mg/l in December, 1988, a 243-fold increase(9) in nine months. By the end of 1989, DBM collected from five different regions showed varied chlorfluazuron resistance as the LD_{50}s were 61.8-1,685.7mg/l, while the LD_{50}s to teflubenzuron ranged from 100.5 to 72,628.6 mg/l(9).

The cross resistance within the BPU-type IGR group is rather obvious. Although hexaflumuron and flufenoxuron have never been registered in Taiwan, resistance to both insecticides had developed in three field DBM strains and two IGR-resistant strains selected by chlorfluazuron and teflubenzuron(6).

The IGR resistance was significantly but not entirely affected by the action of piperonyl butoxide. The synergistic ratios (S.R.) for teflubenzuron were 7.9-10.4 in three DBM populations, while the S.R. for chlorfluazuron in five IGR-resistant DBM strains were in the range of 2.6 to 16.3(9). Without selection pressure, the IGR-resistance gradually declined in DBM, for example, after 17 selection-free generations the resistance ratios of Lu-chu strain declined from 7,621 and 243 to only 6.5 and 3.1 for teflubenzuron and chlorfluazuron, respectively(9).

By crossing with the susceptible strain, the IGR resistance in DBM reduced drastically. Such a genetic manipulation can result in greater reduction in IGR resistance than the synergistic action of piperonyl butoxide. The IGR resistance almost diminished completely in the offsprings of RxS populations in three generations, as the sensitivities of this F_3 offsprings to both teflubenzuron and chlorfluazuron were almost restored to the level of the susceptible strain(9).

Summary of MFO-involved resistances

With the finding of MFO involvement in IGR resistance, the oxidative detoxication obviously appeared as the most important resistance mechanism in DBM. Chou and Cheng(18) first noticed that DBM can develop a unique carbofuran-resistance, surprisingly, which was different from the resistance to carbaryl and methomyl. Based upon that finding, we had questioned the adequacy of the term "carbamate resistance" (35) in regard to DBM because its obscure definition. Cheng et al.(14) again demonstrated the specificity difference in MFOs to carbamates and synthetic pyrethroids. New lines of MFO for metabolizing IGRs further confirmed the diversity and the importance of MFO in DBM. From the study on carbamates, synthetic pyrethroids, IGRs and an experimental insecticide, SN 72129(11), the characters of MFO-involved resistance in DBM could be outlined as follows :

1. Naturally inherent MFO in DBM was responsible for the tolerance/resistance to certain insecticides such as carbaryl, methomyl and propoxur, and can be further selected to a higher resistance level.
2. MFO-involved resistances to carbofuran, synthetic pyrethroids and IGRs were induced.
3. MFOs were highly diversified for different xenobiotics.
4. MFOs were either specific for a single compound, such as carbofuran, or an entire

chemical group such as synthetic pyrethroids.

5. MFOs involvement was genetically diversed, being recessive for IGRs, intermediate for synthetic pyrethroids, and dominant for carbamates.
6. The MFO-involved resistances responded indefinitely in amplitude and could eventually render the control chemical ineffective at any dosage. Carbaryl, methomyl, carbofuran, synthetic pyrethroids and IGRs have all met the same fate in DBM control.

All these facts indicate that DBM has a very efficient, active, both inherent and inducible MFO complex. Whenever a xenobiotic molecule fits to be the substrate for one of the many "hidden" MFOs, the survived DBM with this subline MFO would multiply under the selection, and a new MFO-involved resistance would appear.

(4) Resistance to organophosphorus insecticides

DBM resistance to organophosphorus insecticides (OPs) is characterized by a series of complicated cross resistance patterns; each covers only a limited number of OPs, and was dependent upon the initial and subsequent selectors. Cheng et al.[8] had characterized the OP-cross resistance by selecting DBM with mevinphos, profenofos and prothiophos. Similar selections have been done for cyanophos, dichlorvos, prothiophos[33], diazinon[17] and phenthoate[28]. Mevinphos induced only slight and unstable resistance (R.R.=8.0 after 20-generation selection) with limited cross resistance to other OPs[7]. According to Sun et al.[35], the resistance declined faster in regard to mevinphos than profenofos and prothiophos. Dichlorvos-selected DBM developed strong resistance to cyanophos, dibrom, salithion, mecarbam and fenitrothion, but showed little or no resistance to other 11 OPs. Sasaki indicated that prothiophos-selected DBM developed strong resistance to 10 out of 17 OPs tested[33], while we observed prothiophos- and profenofos-selected strains only developed resistance to 3 out of 8 OPs[8].

Cheng et al.[5] summarized the OP-resistance in DBM into three groups according to the resistance stability in field:

Group Ⅰ: The least stable group includes mevinphos, phenthoate, methidathion and pirimiphos-methyl. The developed resistances decayed within 1-3 months.

Group Ⅱ: The intermediate stable group includes profenofos and prothiophos. The resistances of this group can last for about 3-6 months.

Group Ⅲ: The most stable group is the resistances to methamidophos, mephosfolan and quinalphos, and the resistances to these insecticides can persist for more than 6 months.

Responses similar to those field observations were confirmed in the laboratory. For instance, DBM with the least resistance to mevinphos, phenthoate and methidathion, turned out to be strongly resistant to methamidophos, mephosfolan and quinalphos[8,14,23]. The stability difference in OP resistance is probably a major reason why certain OPs are still effective for DBM control. Hence they should be considered in the alternate spray schedule.

(5) Resistance to cartap

In Japan[32] and Taiwan[4], DBM developed only weak resistance to cartap after prolonged field usage. However, in the Cameron Highlands of Malaysia, cartap resistance became very strong[34] and the high resistance to cartap can also be induced in the laboratory[18].

The mechanism of resistance to cartap is still unknown. The F_1 offsprings of crossing between resistant and susceptible strains had higher cartap-tolerance than either parent strain. In the isoelectric focusing(IEF) observation, the esterases of F_1 offsprings had higher activities and more isozymes[12], and it was suggested that broad-spectrum esterases in DBM enhance its tolerance to cartap by hydrolysing the thioester bond. The cross resistance to cartap had been reported in a mevinphos-[7], two phenthoate-selected strains[28], and a minor cross resistance occurred in a fenvalerate-resistant strain as well[18]. Surprisingly, mevinphos, phenthoate and fenvalerate all have an ester bond in their structures.

On direct contact, cartap has a good initial action against DBM, but its residual effect is weak[23] because the highly soluble cartap can be washed off by the moisture condensed on leaves in the evening.

(6) Resistance to *Bacillus thuringiensis* (B.t.)

Commercial B.t. products have beeen introduced and widely used in Taiwan for more than 30 years. Field evaluation indicated that new B.t. formulations, viz. SAN415, Florbac-FC, Florbac-XLV and CGA237218 were better than the first generation B.t.[24], and the possibility of B.t. resistance was suspected. However, study in 1992 to 1994 on B.t. efficacy in field DBMs confirmed the existence of B.t. resistance. Thuricide with the longest marketing history in Taiwan, exhibited highest resistance ratio(R.R.) at 118.3. The estimated R.R. of field DBM to *B.t.* subsp. *kurstaki* was 5.4-118.3, and 34.9 to the single CryIA(c) toxin product, MVP. Mild cross resistance of *B.t.* subsp. *kurstaki* to *B. t.* subsp. *aizawai* was also detected[21].

Test of the laboratory-maintained field colonies showed that the B.t. resistance ratios were merely 1.3-7.0, indicating the B.t. resistance will decline in DBM if they are relieved from the selection. Field DBMs collected freshly from different regions showed various insensitivities to B.t. products, reflecting regional difference of B.t. usage in the field[21].

Many efforts have been made to understand the molecular basis of selectivity and insecticidal properties of B.t. toxins. A brief overview of B.t. structures, the interaction of B.t. toxins with insect cell membranes, the histopathology of B.t. action, and a discussion of insect resistance to B.t. was reviewed in 1992[20].

2. Resistance alert for newly introduced insecticides

(1) Diafenthiuron

Diafenthiuron was registered for DBM control in Taiwan since 1993, which has a better and steadier control efficacy on both DBM and *Spodoptera litura* as compared to newly developed B.t. products[24]. Laboratory tests showed diafenthiuron confronts with no cross resistance from other existing insecticides, and PB had no effect on the

efficacy of diafenthiuron[10].

(2) Abamectin

Abamectin was registered in Taiwan for DBM control in 1992-93. Although its control efficacy for resistant DBM is excellent, the acceptancy by farmers is affected by higher cost than other insecticides. Following one year field application, minor abamectin resistance has been detected in two field DBM populations, Lu-chu and Erlung, and the R.R. were 32 and 27, respectively, compared to TARI's susceptible IL strain[22]. The LD_{90} for both field strains was 2.0-3.0 mg/1, which is still lower than the field-recommended spray dosage of 10 mg/1, hence the farmers may not notice the loss in control efficacy. Continuous monitoring of abamectin resistance will be conducted to determine whether this pesticide can be adopted into further insecticide alternation program.

3. Selection of insecticides for alternate spray strategy

For alternate spray, selection of insecticides with independent mode of action is the most important prerequisite because the influence of cross resistance can be minimized. Cartap, B.t. and OPs are the proper candidates, while carbamates, synthetic pyrethroids and IGRs should not be included in the alternate spray program due to the readily induced MFO activities. For OPs, only those confront with inconstant and moderate resistance (e.g., mevinphos, phenthoate, methidathion and pirimiphos-methyl, etc.) should be considered for rotation, and among them mevinphos is the most favorable choice for its short residual span.

We have constructed a list of selected insecticides and their correspondent target pests (Table 2) as the field instruction material for farmers.

Table 2. Insecticides selection for managing resistant DBM and other crucifer pests

Insecticides	Dilution	DBM[z]	CPR	CWW	ICW	CLP	BAW	TCW	FB	APH	LM
Mevinphos	500X	+[y]	+	+	+			+	+		
(or Profenofos)	1000X	+	△	△	△			△	+		
B.t.	2000X	+	+	+	+	+					
Cartap	1000X	+								△	△
Bifenthrin	1000X		△	△	+	+	+	+		+	
Cyromazine	6000X										+

z DBM--diamondback moth ; CPR--cabbage pyralia ; CWW--cabbage webworm ; ICW--imported cabbage worm ; CLP--cabbage looper ; BAW--beet armyworm ; TCW--tobacco cutworm ; FB--stripped flea beetle ; APH--aphids ; LM--leaf-miner

y + : registered for pest control △ : not registered but effective for control

For other pests, one synthetic pyrethroid is included due to its mammalian safety and broad control spectrum for pests such as *Spodoptera litura, S. exigua, Trichoplusia ni,* etc. The SP-resistance induced in DBM will not interfere with the selected control agents for the sake of no cross resistance.

So far DBM has developed mild resistance to abamectin, and no evidence for resistance to diafenthiuron. This makes them appropriate as the independent DBM control agents. If they meet the requirement of no cross resistance to other insecticides, they will serve as future candidates in the alternate spray program.

4. Other considerations in insecticide selection

In practice, culture-related factors as well as other important crucifer pests should be considered in insecticide selection :

(1) Other important pests include imported cabbage worms (ICW, *Pieris rapae crucivova* and *P. canidia sordida*), tobacco cutworm (TCW, *Spodoptera litura*), beet armyworm (BAW, *Spodoptera exigua*), cabbage webworm (CWW, *Hellulla undalis*), cabbage pyralia (CPR, *Crocidalomia binotalis*), cabbage looper (CLP, *Trichoplusia ni*), stripped flea beetle (FB, *Phyllotreta striolata*), leaf-miner (LM, *Liriomyza trifolii* and *L. bryoniae*), and the aphids (APH, *Aphis gossypii, Lipaphis erysimi* and *Brevicoryne brassicae*).

(2) Profenofos should be used to substitute mevinphos in DBM control when stripped flea beetle is also a concern in the field.

(3) Different B.t. products have to be evaluated against DBM for B.t. resistance, especially for new B.t. product.

(4) For farmers frequently grow other leafy vegetables adjacent to crucifers, precaution should be made to cartap usage as it can cause phytotoxicity in the young seedlings of those vegetables.

(5) Resistant *Liriomyza trifolii* invaded Taiwan in 1988, and we are not able to control it by either biological or other non-chemical agent so far. When the leaf-miner population density is low, cartap and thiocyclam hydrogenoxalate are recommended, and cyromazine should be used only at high leaf-miner density. Besides, leaf-miner can also be controlled by abamectin.

5. Constraints in implementing RMP in Taiwan

In Taiwan, farm unit is small and usually intensively cultivated. Crop varieties are numerous. The hot and humid weather favors the pests and diseases. Agricultural manpower is either aged or in shortage. All these factors make the implementation program significantly different from the resistance management for large acreage crop, e.g. cotton in Australia(19).

Several significant constraints are identified as follows :

(1) Through a 40-year longterm practice, reliance on chemical insecticides is both psychological and operational. Preventive spray has become the best policy adopted by farmers when dealing with the year-round cropping of a great variety of crops. Pest control as a measure of insurance has become routine operation in farming.

(2) In Taiwan, pesticide industry operates in a free market, and the competition of retailers caused a lot of misuses of unfit pesticides in dealing with the resistance problem.

(3) Instead of using insecticides rationally through the recommendation of extension

service, mixing more insecticides in one spray has become a very common solution for relieving the resistance. This ill-fated practice was further encouraged and intensified by pesticide retailers.

(4) All categories of insecticides are used in the same region during the same period, which render the coordination of an area-wide insecticide alternation schedule extremely difficult.

(5) In Taiwan, the warm and humid environment promotes the generation overlapping of pests. Thus any resistance management strategy on seasonal or window-type[19] basis is impractical and destined to failure.

MATERIALS AND TACTICS FOR IMPLEMENTATION

1. Insecticides are supplied to nucleus farmers by "the package consideration"

In order to grow good quality crucifers, vegetable farmers have to control both the resistant DBM and other pests. If only aimed at the resistant DBM, the selected insecticides may not provide satisfactory control for other pests, and farmers will have no choice but add other insecticides. This will cause (1) interference in insecticide alternation, and (2) confusion in interpretating the final implementation results. Therefore, insecticides for controlling other insect pests were also included in implementation.

Based on the rationales of efficacy, resistance and cross resistance, a complete package of 6 insecticides was selected for all major pests in Table 2. The insecticides were supplied to those participated nucleus farmers at a 3-month interval and only at demonstration quantity.

2. Notes for insecticides usage on major crucifer pests :

DBM

(1) For avoiding resistance development, OP, cartap and B.t. should be used alternately.

(2) Bifenthrin is for pests other than DBM, since DBM has already developed SP-resistance.

(3) During high DBM density period, from October to Feburary, spray interval may be shortened to 5 days if necessary.

(4) B.t. spray can also control BAW and CWW.

Other lepidopterous pests

(1) If many species of lepidopterous pests occurred at the same time, do not use many insecticides, try the broad spectrum bifenthrin first.

(2) Bifenthrin is also effective against the eggs of TCW and BAW[16].

(3) Mevinphos and profenofos are effective against DBM, CPR, CWW and TCW, and can reduce the use of bifenthrin.

(4) B.t. is also effective against some lepidopterous pests.

Flea beetle (FB)

(1) The eggs and larvae of FB are in the soil. Larvae feed on roots and adults on leaves.

(2) When there is an outbreak, a 3-5 days spray interval is needed.

(3) Flooding the field before planting is very helpful.

(4) Profenofos is more effective than mevinphos.

Leaf-miner (LM)

(1) When leaf-miner density is low, cartap can provide enough protection.

(2) Cyromazine should be used to suppress the outbreak of leaf-miner.

Aphids

Mevinphos, cartap or bifenthrin can offer proper protection, but they should be used alternately.

3. Examples of insecticides rotation for various pest conditions :

Case 1 : DBM+other lepidopterous pests

Use the following combination alternately :

(1) mevinphos+bifenthrin

(2) cartap+bifenthrin

(3) B.t.+bifenthrin

Case 2 : DBM+FB

Use profenofos or the following combination alternately :

(1) profenofos+B.t.

(2) profenofos+cartap

Case 3 : DBM+LM

Alternately use :

(1) cartap

(2) mevinphos+cyromazine

(3) B.t.+cyromazine

Case 4 : DBM+aphids

Alternately use :

(1) cartap

(2) mevinphos

(3) B.t.+bifenthrin

4. Role of farmer organization : training and on-site education

Farmer association is responsible for coordinating nucleus farmers in the implementation program, and TARI's specialists visit farmer associations once a month on the following occasions :

(1) Supplying selected insecticides.

(2) Holding discussion session with farmers.

(3) Teaching the farmers to identify pests.

(4) Discussing the advantages and disadvantages of suggested control measures on efficacies, residues, safety and cost.

(5) Collecting the implementation working records, and commenting on erroneous usage of insecticides.

5. Report session of participated farmers to their neighboring farmers

At the end of the year, every participated farmer is obligatory by contract to give a talk about his experience to the neighboring farmers in the training sessions.

6. Providing the well-illustrated leaflet of both major crucifer pests and insecticides for rotation application to all vegetable farmers.

We found that vegetable farmers are extremely lacking the knowledge of pest identification and insecticides selection. To solve this problem, a brochure of "Resistant Pests Management on Crucifer Vegetables" was edited and issued in 1993 as a training material to participated farmers.

RESULTS AND DISCUSSION

1. The advantage of supplying a complete insecticide package to participated farmers was manifested in the final evaluation. Participants apparently had a better knowledge about each recommended insecticide and its target pests. A lot of experienced farmers were surprised to find out that the effectiveness of rationally selected insecticide is equivalent to or better than their own choice of mixing four or five insecticides. The most significant improvement is that farmers have learnt to select insecticides on a more informative basis. In the field discussion sessions, nucleus farmers become awakened and realized their 20 to 30 years experience of mixing insecticides is not a correct solution for dealing with pesticide resistance. The fact also revealed that serious DBM resistance problem was probably due to the use of many unfit insecticides.

2. The participated farmers become more confident and enthusiastic as they become volunteer instructors for the implementation program. Neighbors of the nucleus farmer could easily verify his RM practice. Besides, this "honor system" tactics ensured the participated farmer to conduct the experiment truthfully with only minimal manpower for supervising.

3. The brochure of resistant pests management on cruciferous vegetables has gained well acceptance. In view of the response of farmers, the Department of Agriculture and Forest of the Provincial Government immediately adopted the brochure as the official extension material, and 65,000 extra copies were printed for dissemination.

4. An evaluation of this RMP was conducted after implementing the program for two years (1990—1992) on the insecticides mixing, the spray interval, the insecticide expenditure as well as the benefit. In 1991—92, 65 farmers belonging to 8 farmer's associations have gained an average of 20% increase in crop income, 32% saving in insecticide expenditure, 2.3 days' lengthening of the spray interval (from 5.0 days to 7.3 days) and 1.8 insecticides reduction in tank mixture (from 5.1 to 3.3 per tank) (Table 3). The result of 1992—93 was similar, 127 farmers of 21 farmer associations have gained a 15% increase in crop income, 37 % saving in insecticide expenditure, 2 days' lengthening of the spray interval (from 6.0 days to 8.0 days), and 1.5 insecticides reduction in tank mixing (from 4.0 to 2.5 per tank) (Table 3).

5. Newly introduced DBM control agents e.g. diafenthiuron, new types of B.t. and abamectin, will attract the farmer's attention away from the resistance management practice for the reason of convenience rather than the cost. But following the recent finding of DBM resistance to B.t. and abamectin, unavoidable consequence will make the resistance manage-

ment more prevailing in the future.

Table 3. Evaluation of implementation program for the management of pesticide-resistant DBM and other crucifer pests in 1991—93

Evaluation Item	1991—92[z]	1992—93	Mean
Farmer's appraisal (%)			
Good	61.5	56.0	57.8
Fair	37.0	44.0	41.7
Poor	1.5	0	0.5
Insecticide saving (%)	32.0	37.0	35.0
Spraying interval (days)			
Before implementation	5.0	6.0	5.7
After implementation	7.3	8.0	7.8
Insecticides/Tank mixture			
Before implementation	5.1	4.0	4.4
After implementation	3.3	2.5	2.8
Income increase (%)	20.0	15.0	16.7

[z] In 1991-92, there were 65 farmer participants belonging to 8 farmer associations (FA), while in 1992-93, 127 participants from 21 FA.

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抗藥性小菜蛾及其他十字花科蔬菜害蟲之管理[1]

鄭 允[2] 陳秋男[3] 高靜華[2]

摘 要

十字花科蔬菜重要害蟲，小菜蛾（*Plutella xylostella* L.），因抗藥性問題而難以防治，經究明其抗藥性及藥劑間之交互抗性關係，確定應該避免使用易爲多功能氧化酵素（MFO）分解之對象藥劑如氨基甲酸鹽、合成除蟲菊精及昆蟲生長調節劑等，而採用培丹、蘇力菌及抗性不穩定之有機磷類，如美文松、亞特松及滅大松，輪流使用，即可達到防治並消除抗性干擾之目的。工作中並將十字花科蔬菜其他重要害蟲，如大型鱗翅目害蟲、黃條葉蚤、潛蠅及蚜蟲等之防治藥劑一併考慮，而提出解決抗藥性小菜蛾及其他害蟲用藥之說明及範例，供農民使用。經過三年田間之示範，並評估29個參加農會之192名參試農民，結果顯示此一綜合防治用藥較農民慣用技術平均可減少35％農藥支出；噴藥間隔可由5.7天延長爲7.8天；藥劑之混合使用由4.4種減至2.8種；而蔬菜農友之收入也增加了16.7％，因而此一防治策略及技術已被納入正規推廣系統，成爲本省十字花科蔬菜害蟲之藥劑防治規範。

關鍵詞：小菜蛾、十字花科蔬菜害蟲、抗藥性管理策略、推廣、核心農民。

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2. 本所應用動物系研究員及助理研究員。臺灣省 臺中縣 霧峰鄉。
3. 行政院農委會農糧處植物保護科科長。臺北市。

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Effects of Simulated Acid Rain on Pollination of Rice Plants[1]

Ming-Hwi Yao,[2] Shu Chen[3], Jin-Chuan Tsai[2] and Lien-Sheng Liang[4]

Abstract: This study was conducted to determine the effect of simulated acid rain of pH 3.0, 4.0 and 5.6 on pollen grain germination and yield in rice (*Oryza sativa L.*) plants. Results indicated that direct exposure to acid rain of pH 3.0 and pH 4.0 acid rain reduced pollen grain germination by up to 30% and 10%, respectively. These alternations closely paralleled to decreasing in seed setting, number of spikelets per panicle and grain yield. When exposed to pH 5.6 acid rain, rate of pollen grain germination decreased, but the effect was not significant. Germination rate of pollen grown on agar medium at pH 4.0 was 54% of that grown on pH 5.6. At pH 3.0 the pollen graims almost did not germinate. The two rice varieties (Tainung 67 and Taichung sen 10) had similar pollen grain germination rate both *in vivo* and *in vitro* in response to acid rain.

Key words: Acid rain, Rice (*Oryza sativa L.*), Pollen grain germination, Yield components.

INTRODUCTION

Nitrogen and sulfur oxides emitted into the atmosphere may react with atmospheric moisture to form acidic solutions. Natural rain water, in equilibrium with atmospheric CO_2 has an approximate pH value of 5.6. As a result of increased pollutant levels, the annual average pH value of precipitation in Taiwan is 4.86, with individual rainfalls as low as 3.8 [(3)]. The oxidation and hydration of oxides of sulfur and nitrogen from anthropagenic sources attributed to the increased acidity in precipitation.

The effects of the deposition of acidic substances on plant growth and development are not well understood. The acidity of simulated rain may increase, have no effect upon, or decrease crop yield[(8,12)]. Assessing the effects of general atmospheric pollutants and particular acid rain on agricultural and forest ecosystems will be difficult until the physiological and developmental mechanisms involed in plant response to atmospheric deposition are better understood.

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1. Contribution No. 1835 from Taiwan Agricultural Research Institute.
2. Respectively, Assistant, Associate Researcher, and Head, Department of Agricultrual Engineering, TARI, Wufeng, Taichung, Taiwan, ROC.
3. Assistant, Department of National Plant Gentic Resources Center, TARI, Wufeng, Taichung, Taiwan, ROC.

Acid conditions are known to have adverse effect *in vitro* on germination and growth of pollen from several plants[1,3,16]. Forsline et al. (1983) reported that simulated rain pH below of 2.75 inhibited *in vitro* pollen germination in apple. In studying the effects of acid rain on pollen germination *in vivo,* Cox (1984) observed that pH 3.6 simulated rain applied before pollination to the stigma of evening primrose decreased subsequent pollen germination. Wertheim and Craker (1988) noted that germination of corn pollens was reduced on silks treated with simulated acid rain. Both of these observations suggest that acid precipitation falling on exposed stigma may hinder pollen-silk interaction and /or the development of pollen tube required for fertilization of an egg cell. This reduced the number of fertilization and resulted in decreasing of seeds.

The objectives of these experiments were to determine if acid rain event on the flower would affect seed setting and grain yield in rice and if any yield reduction was related to the quantities of pollen used in the pollination process.

MATERIALS AND METHODS

Acid treatments:

The pH value of the simulated rain was adjusted to one of the three levels (pH 5.6, 4.0 or 3.0) with sulfuric and nitric acids at a constant sulfate/nitrate ratio of 2.5: 1 on an equivalent basis. Acid solution in a PVC supply canister was pressurized with hand and forced through a small orifice of a commercial stainless steel rain nozzle. Where it impinged on the tip of a J-shaped pin and was broken into particles with mean stain diameter of 1.0 mm. Spraying was carefully done to wet the panicles surface evenly and completely.

Under normal weather conditions in Tawian, most rice varieties begin anthesis at about 0900 and end at about 1100 hour. On rainy days, pollination may occur without the opening of the lemma and palea. For this reason, exposure were conducted at 0800 (before flowering) and 1000 (flowering) hour, to determine the indirect and direct exposure of pollen to simulated acid rain applied on the processes of sexual reproduction of rice. At the beginning of anthesis, simulated rain was delivered to the plants five times a week until the conclusion of the flowering.

Greenhouse study:

Rice seeds ("Tainung 67" and "Taichung sen 10") were sown in plastic pots (3 seeds a pot, pot size: 1/5,000 ha) filled with 2L of loamy soil. Seedlings were thinned to one plant per pot 7 days after sowing. The experimental plants were allocated in a greenhouse that served as an ambient rain shelter for the delivery of simulated acid rain.

In vivo pollen grain germination experiment:

A subsample of five spikelets was removed from the top three primary branches, placed in 3: 1 v/v basis solution of absolute ethylalcohol and acetic acid fixative. Stigmas from sample spikelets were excised and placed on a glass slide, then stained with acetocamine dye. Using an optical microscope, the total number of pollen grains on stigmas and number of germinated pollen grains were counted. Pollen viability was computed as the ratio of germinated pollen grains to total pollen grains on a stigma.

Field study

Field study was conducted to determine the effects of simulated acid rain on yield and yield components of rice plants when treated at the anthesis. Two rice cultivars (Tainung 67-japonica and Taichung sen 10-indica) selected to represent a diversity of agronomic characters were used in the experiment designed to identify the damage by simulated acid rain treatment. The experiment was conducted on the experimental farm in the first and second crop seasons 1994. Seeds were sown using wetbed technique and one month-old seedlings were transplanted to a plot (5.0^{*} $2.0m^2$) with 1 plant/hill. Each plot contained seven 5-m rows of rice plants spaced 0.2-m apart. The layout was a randomized complete block design (RCBD) with 3 replications. Fertilizer was applied at the rate of 60-40-40 kg N-P_2O_5-K_2O per hectare before transplanting. Weeds were controlled with occasional hand weeding. Insecticides were applied as often as necessary.

***In vitro* pollen grain germination experiment:**

The preparation of the agar medium followed the method of Kariga (1989). 1% agar and 20% sucrose were dissolved in heated distilled water (pH adjustment, pH 5.6, 4.0 and 3.0) and the solution was cooled to about 60°C with stirring in a waterbath. 20ppm $K_2B_4O_7$ were added immediately into the agar and sucrose medium. The prepared medium was poured into petri dishes (4.5 cm in diameter) and the surplus was removed in order to make a thin layer. The germinated pollen grains were counted under a microscope about 20 min after shedding of pollen grains on the medium. Any pollen grains with a tube more than a half of the grain diameter were regarded as germinated. In each experiment, 200-300 pollen grains were counted.

RESULTS

Pollen grain germination was reduced on stigma under direct exposure to the simulated rain of pH 3.0 as compared with germination on stigma under direct exposure to the simulated rain of pH 4.0 and pH 5.6 (Table 1). However, there were no significant differences of indirect exposure to acid rain on all the germinating characters of pollen.

Germination rate of the two cultivars on agar medium at pH 4.0 were both near 54% of that grown on medium at pH 5.6 (Table 2). At pH 3.0, the pollen grains almost did not germinate.

For direct exposure, the different pH treatments did not significantiy affect the number of panicle per hill and 1,000 grain weight for both cultivars. The average percentages of seed setting of panicles which was directly exposed to pH 3.0 rain 6 times were inhibited were 35.2% in the first crop (Table 3). and 39.6% in the second crop (Table 4). of Tainung 67 in 1994. Similar results were found in Taichung sen 10 (Table 3 and 4). In addition, the seed setting of first crop for Tainung 67 and Taichung sen 10 at pH 5.6 was significantly lower than the no rain treatment (control), however, two cultivars treated with pH 5.6 rain was not significantly lower than control, but, average lower was found in second crop, indicating that pollination process is unable to resist wetting. The yield of both cultivars at the pH 3.0 treatment was significantly less than average yield of the other three treatment. In 4.0

treatment, yield of Taichung sen 10 tended to be less than the pH 5.6 or no rain treatment, whereas the pH 4.0 yield for Tainung 67 was not significant. However, the yield components of two in which panicles were cultivars indirectly exposed to different acid rain treatments were unaffected.

Table 1. Means of pollination characteristics of Tainung 67 and Taichung sen 10 rice cultivars by simulated acid rain at anthesis stage

		Direct			Indirect		
	no rain	5.6	4.0	3.0	5.6	4.0	3.0
Tainung 67							
pollen grains/stigma (no.)	98.6a	73.3b	76.3b	73.5b	101.7a	95.6a	91.5a*
germinated pollen grains/stigma(no.)	88.6a	59.3b	58.3b	45.9c	88.1a	81.9a	80.3a
In vivo pollen grain germination(%)	89.9a	80.9ab	76.4b	62.4c	86.6a	85.7a	87.8a
spikelets with 0-10 germinated pollen grains/stigma (%)	0.0d	3.7c	15.4b	34.6a	0.0d	0.0d	0.0d
spikelets with >10 germinated pollen grains/stigma (%)	100.0a	96.3b	84.6c	55.4d	100.0a	100.0a	100.0a
unfilled bloomed spikelets (%)	11.6a	18.8ab	7.2b	41.6c	9.8a	12.3a	14.8ab
Taichnung sen 10							
pollen grains/stigma (no.)	84.6a	77.4a	58.6b	65.3b	82.0a	83.2a	79.6a
germinated pollen grains/stigma(no.)	76.4a	67.4b	7.8c	49.5c	77.2a	19.1a	72.1a
In vivo pollen grain germination(%)	90.3a	87.1ab	81.6b	75.8c	94.1a	95.0a	90.6a
spikelets with 0-10 germinated pollen grains/stigma (%)	0.0c	0.0c	8.7b	24.2a	0.0c	0.0c	0.0c
spikelets with >10 germinated pollen grains/stigma (%)	100.0a	100.0a	91.3b	75.8c	100.0a	100.0a	100.0a
unfilled bloomed spikelets (%)	9.6a	10.3a	17.8b	30.8c	9.2a	9.5a	10.6a

* Means in each row with the same letter are not signficantly different at 5% level (Duncan's New Multiple Range Test)

Table 2. Means of pollen grain germination rate (%) of Tainung 67 and Taichung sen 10 rice cultivars under different pH of culture medium *in vitro*.

	Medium pH		
	5.6	4.0	3.0
Tainung 67	92.5a	51.3b	5.6c*
Taichnung sen 10	90.6a	48.6b	3.7c

* Means in each row with the same letter are not signficantly different at 5% level (Duncan's New Multiple Range Test)

Table 3. Yield and yield components of Tainung 67 and Taichung sen 10 in simulated acid rain treatments in the first crop of 1994.

	no rain	Direct 5.6	Direct 4.0	Direct 3.0	Indirect 5.6	Indirect 4.0	Indirect 3.0
Tainung 67							
panicle/hill (no.)	9.6a	9.7a	9.0a	9.3a	9.5a	9.0a	8.9a*
spikelet/panicle (no.)	107.6a	97.4a	86.7b	82.3c	104.3a	101.2a	97.4a
seed setting (%)	95.2a	83.2b	76.6c	64.8d	95.6a	90.6a	94.1a
1,000 grain wt (g)	24.6a	25.1a	23.6a	21.9a	24.1a	24.3a	22.6a
yield (g/hill)	29.7a	28.4a	26.5a	27.1b	30.6a	29.1a	27.8a
Taichung sen 10							
panicle/hill (no.)	11.6a	10.7a	11.2a	10.6a	12.0a	11.4a	11.6a
spikelet/panicle (no.)	100.6a	94.3a	80.9b	73.4c	98.1a	99.8a	90.1ab
seed setting (%)	89.6a	82.0b	69.4c	59.6d	87.5a	90.6a	90.1a
1,000 grain wt (g)	23.6a	22.1a	21.8a	21.8a	23.0a	23.6a	22.7a
yield (g/hill)	30.1a	28.6a	25.3ab	19.4b	30.1a	29.6a	25.7ab

* Means in each row with the same letter are not significantly different at 5% level (Duncan's New Multiple Range Test)

Table 4. Yield and yield component of Tainung 67 and Taichung sen 10 in simulated acid rain treatments in the second crop of 1994.

	no rain	Direct 5.6	Direct 4.0	Direct 3.0	Indirect 5.6	Indirect 4.0	Indirect 3.0
Tainung 67							
panicle/hill (no.)	9.1a	8.8a	9.0a	9.1a	8.8a	8.6a	8.9a*
spikelet/panicle (no.)	98.2a	99.8a	94.0a	91.1a	97.5a	100.2a	98.2a
seed setting (%)	92.8a	84.3ab	79.1b	60.4c	93.7a	89.4a	83.5a
1,000 grain wt (g)	25.2a	24.6a	24.7a	22.5a	23.8a	22.5a	24.6a
yield (g/hill)	25.4a	23.6a	22.7a	16.4b	26.1a	25.0a	20.6a
Taichung sen 10							
panicle/hill (no.)	11.2a	10.5a	9.5a	10.2a	10.8a	11.6a	11.0a
spikelet/panicle (no.)	94.6a	93.0a	79.6b	67.1c	93.2a	91.3a	89.4a
seed setting (%)	87.9a	84.5a	74.4b	66.3c	84.4a	82.9a	81.7a
1,000 grain wt (g)	22.5a	23.1a	21.6a	21.7a	22.1a	21.8a	23.2a
yield (g/hill)	27.9a	25.4ab	22.6b	19.5c	28.2a	27.6a	23.0a

* Means in each row with the same letter are not significantly different at 5% level (Duncan's New Multiple Range Test)

DISCUSSION

Acid deposition can negatively affect crops reproduction in many ways. For example, acid deposition can alter physilogical processes, such as photosynthesis; cause visible injury to leaves, such as chlorosis, necrosis, and premature leaf fall. The effects of acid rain on photosynthesis and leaves may be indirectly responsible for the reduction of seed production. Reproduction in rice and most other plants requires the germination and growth of pollen on stigmatic tissue. Any environmental stress that limits the ability of the male

gametophyte to germinate and grow on the stigma could reduce seed production and could alter the gene pool, especially in natural ecosystems with changes in gametophytic competition[13].

The effects would be more severe if acid rain occurred during pollen grain germination stage. Acid rain of pH 4.0 or less would seriously inhibit germination as well as growth of the pollen tube. Several authors have reported that rain of similar acidity (pH values 3.0-4.0) have inhibitory effects on specific phases of plant growth and fruit setting in tomato[11], adversely affected fruit production in term of individual fruit weight, fruit set and fruit appearance in apple[14]. Results of the study demonstrated that in rice, pollen grain germination was reduced when stigma and pollen grains directly exposed to simulated acid rains of pH 4.0 and 3.0 as compared to a "non-rain" control. At pH 5.6, pollen grain germination was decreased, but not significant. In addition, acid solution had a negative influence on the total number and percentage of germinated pollen grains *in vivo* (Table 1). The deleterious effects on pollen shedding and germination indicate that lack of germination is partly due to a decrease in the number of pollen grains, particularly fertile pollen grains. In pH 3.0 treatment, more than 30% of spikelets had less than 10 germinated pollen grains, It is noted that at least 10 germinated pollen grains per stigma are necessary for successful fertilization[7].

The reasons for reduced pollen grain germination on rice directly exposed to acid rain may be due to chemical or physical effects of acid rain on the stigmatic surface, creating an environmental inhospitality to pollen, or to a direct effect of rain residue on either the pollen grain or elongation pollen tube. However, when pollen grains reach the stigmatic surface, they imbibe water and stigmatic exudation and release enzymes[15]. The presence of toxic compounds or abnormally high acidity may affect their ability to germinate.

Acid rain of pH 3.0 almost totally inhibited pollen grain germination in agar medium culture (Table 2). whereas *in vivo* pollen grain germination (Table 1). and seed settings of field studies (Table 3 and 4) at pH 3.0 were produced a lower 30-40% compared with pH 5.6 or no rain treatments difference. For this result, simulated acidic rain at pH 3.0 may be sufficiently acidic to cause direct damage to and consequent leakage of basic solutes from stigmatic cells[5], such leakage might then buffer surface acidity, may explain the difference of pollen grain germination between *in vivo* and *in vitro* treatments. Karnoaky and Stairs (1974) suggested, based on his work *in vitro* and on experiments with *Populus tremuloides* Michx. (trembling aspen) seed set, that pollen grain germination and tube elongation may be more sensitive when fumigated *in vitro* than when fumigated *in vivo*. He was also proposed that the difference might be due to the greater pH buffering capacity of stigmatic tissues as compared to agar media.

The 10% decrease in seed setting caused by applying pH 5.6 simulated rain after pollination as compared to the no-rain and indirect exposure treatments could be due to wash away of ungerminated but still viable pollen from the stigma. Wet, damp, or humid conditions have been observed to inhibit successful pollen function[4], and even simulated rain without adding pollutants may have similar effect.

The adverse effects of single episodes of simulated acid rain reported here and elsewhere [3,17] demonstrate the potential for acute effects of atmospheric deposition on flowering and sexual reproductive processes should be considered when studying the influence of atmospheric pollutants on plant growth, development, and yield in agricultural and forest ecosystems.

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人工模擬酸雨對水稻授粉之影響[1]

姚銘輝[2] 陳述[3] 蔡金川[2] 梁連勝[4]

摘 要

本試驗主要探討水稻開花時人工模擬酸雨對花粉發芽的影響，並以田間資料探討影響產量減少的原因。結果顯示，水稻在開花時，直接噴施 pH 3.0及 pH 4.0的酸雨時花粉發芽率分別受抑制30%及10%，另發芽率降低將使穀粒結實率、每穗小花數及產量下降。噴施 pH 5.6酸雨則發芽率略爲下降，但未達顯著水準。若花粉未直接受酸雨噴施，則花粉發芽率及產量皆不影響。另將花粉置於不同 pH 值培養基培養，則酸性抑制花粉發芽的效果更爲明顯。兩個水稻品種(臺農67號及臺中秈10號)的花粉發芽率對酸雨的反應相似。

關鍵詞：酸雨、水稻、花粉發芽、產量構成因素。

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1. 臺灣省農業試驗所 研究報告第 1835 號。
2. 本所農工系助理、副研究員。臺灣省 臺中縣 霧峰鄉。
3. 本所作物種原室助理。臺灣省 臺中縣 霧峰鄉。
4. 本所農工系主任。臺灣省 臺中縣 霧峰鄉。

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